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EDITED BY

SIMON FLEXNER, M.D.

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ETIOLOGY OF YELLOW FEVER.

IV. The Acquired Immunity of Guinea Pigs against Leptospira icteroides after the Inoculation of Blood of Yellow Fever Patients.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 2, 1919.)

Most of the attempts to transmit yellow fever by inoculating the blood of patients into various animals have ended in failure. As stated elsewhere[1] out of 74 guinea pigs, in only eight were the results positive, and in other species of animals they were all negative. It should be emphasized that for the purpose of transmission cases were selected which were still in the early stage of the disease, previous investigators having pointed out the fact that the virus may no longer exist in the peripheral blood after the 3rd day of the illness.

In the present study the guinea pigs which failed to manifest the typical symptoms, or any symptoms at all, were kept under daily observation for many weeks. On a later date, through temporary shortage of normal guinea pigs, they were inoculated, together with several normal guinea pigs, with a virulent organ emulsion, rich in the leptospira, from a guinea pig experimentally infected with the organism, in order to prepare a large amount of organ emulsion for the purpose of immunizing two donkeys against the virus. A great many of the guinea pigs remained perfectly well, while most of the normal animals, as well as those which had been previously inoculated with the blood of malarial patients,[2] came down with an experimental infection.

[1] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 565.

[2] In a country where æstivo-autumnal parasites infest the population many times a malarial patient is brought to the Yellow Fever Hospital on the chance that the case is one of early yellow fever. All these cases were used for the experiments on the transmission of yellow fever without the loss of time which would have been caused by waiting for a diagnosis, for which a day or two might be required.

TABLE I.

Susceptibility of Guinea Pigs to the Inoculation of Leptospira icteroides 25 Days after They Had Been Injected with the Blood of Yellow Fever Patients.

Patient.	Sex.	No. of guinea pig.	Day of disease when bled.	Amount of blood injected.	Reaction.	Subsequent test for susceptibility.	Reaction.	Result.
				cc.	°C.		°C.	
Case 27. Yellow fever. Died in 16 days.	M.	16	14th	2	None.	Virulent Arias strain after 25 days.	40.3 in 5 days.	Killed in 8 days. Typical.
		17	14th	3	"	" "	39.8 " 6 "	Died in 8 days. Typical.
Case 25. Yellow fever. Mild. Recovery. Also malaria.	F.	21	6th	3	"	" "	39.9 " 6 "	Died in 8 days. Typical.
		22	6th	3	39.7 in 4 days.	" "	None.	Remained well.
Case 1. Malaria (Guayaquilian).	"	23		3	None.	" "	40.5 in 8 days.	Killed in 11 days. Mild but typical.
		24		4	"	" "	40.0 " 7 "	Killed in 9 days. Typical.
Case 27. Yellow fever. Second specimen of blood.	M.	32	15th	5	"	" "	39.9 " 5 " Icterus.	Killed in 7 days. Typical.
		33	15th	5	"	" "	40.0 in 6 days. Icterus.	Died in 8 days. Typical.
Case 13. Dr. Valenzuela's patient. Yellow fever. Mild. Recovery.	"	35	3rd	3	39.8 in 8 days.	" "	40.2 in 4 days.	Recovered.
		36	3rd	5	39.2 " 12 "	" "	39.6 " 5 "	Remained well.

Case 2. Malaria.	M.	30		2	None.	Virulent Arias strain after 25 days.	40.4 in 4 days. Icterus.	Died in 9 days. Typical.
" 3. "	"	52		3	"	" "	39.9 in 6 days. Icterus.	Died in 10 days. Typical.
" 4. "	"	19		2	"	" "	40.0 in 9 days. Icterus.	Killed in 12 days. Typical.
		20		2(?)	"	" "	40.0 in 11 days. Slight icterus.	Died in 17 days. Mild but typical.
" 7. Yellow fever. Severe. Recovery.	"	37	6th	5	39.8 in 6 days.	" "	None.	Remained well.
Case 12. Yellow fever. Moderate. Recovery.	F.	39	2nd	5	39.2 " 8 "	" "	39.0–39.7 in 5–6 days.	" "
		40	2nd	3	39.2 " 8 "	" "	None.	" "
Case 5. Malaria.	"	41		3	39.6 " 9 "	" "	40.0–40.3 in 9 days.	Died in 13 days. Typical.
		42		5	39.7 " 6 "	" "	40.5 in 7 days. Icterus.	Died in 10 days. Typical.
" 27. Yellow fever. Autopsy material 6 hrs. post mortem.	M.	48	16th	2 (kidney emulsion).	None.	" "	40.0 in 8 days. Jaundice later.	Recovered (!).
		49	16th	"	"	" "	40.0 in 7 days. Slight jaundice later.	"
Case 21. Yellow fever. Died on 4th day.	"	54	4th	3*	39.4 in 5 days.	" "	None.	Remained well.
		55	4th	3	39.8 " 5 "	" "	"	" "

*Another guinea pig (No. 56) received the same amount at the same time and died in 7 days with suspicious lesions. From it transfers were made into two guinea pigs. Both showed mild fever in 5 days and recovered. The two also resisted the subsequent inoculations of virulent Arias strain.

TABLE I—*Concluded.*

Patient.	Sex.	No. of guinea pig.	Day of disease when bled.	Amount of blood injected.	Reaction.	Subsequent test for susceptibility.	Reaction.	Result.
				cc.	°C.		°C.	
Case 22. Yellow fever. Died in 10 days.	M.	198	5th(?)	3	40.0 in 4 days. Trace of icterus (?).	Virulent Arias strain after 25 days.	None.	Remained well.
		199	5th	3	40.0 in 7 days. Trace of icterus (?).	" "	"	" "
Case 19. Yellow fever. Severe. Recovery.	"	200	4th(?)	3	39.6 in 4 days.	" "	"	" "
		201	4th	3	40.0 " 5 " Trace of icterus.	" "	"	" "
Case 20. Yellow fever. Severe. Recovery.	"	220	4th(?)	3	40.3 in 7 days. Trace of icterus (?).	" "	"	" "
		221	4th	3	40.2 in 5 days. Trace of icterus (?).	" "	"	" "
Case 16. Yellow fever. Severe. Recovery.	"	222	3rd(?)	3	40.1 in 7 days. Trace of icterus (?).	" "	"	" "
		223	3rd	3	40.3 in 6 days. Trace of icterus (?).	" "	"	" "

Case 28. Yellow fever. Died.	M.	329	(?) Brought in unconscious. Died same day.	5	None.	Virulent Arias strain after 25 days.	40.0 in 5 days. Icterus.	Died in 7 days. Typical.
Case 10. Yellow fever. Died in 7 days.	F.	338	2nd	4	"	" "	40.1 in 6 days. Icterus.	Killed in 8 days. Typical.
		339	2nd	4	"	" "	39.5 in 7 days. Icterus.	Killed in 10 days. Typical.
Case 24. Yellow fever. Died.	M.	340	(?) Died next day.	4	39.2 in 5 days. No icterus. Died in 8 days; hemorrhages.			
		341	" "	4	40.0 in 13 days. No icterus.	" "	40.0 in 6 days. Icterus.	Died in 9 days. Typical.
Case 8. Yellow fever. Severe. Recovery.	"	342	2nd	4	40.0 in 12 days. No icterus.	" "	40.2 in 7 days. Icterus.	Died in 10 days. Typical.
		343	2nd	4	39.6 in 6 days. Trace of icterus.	" "	None.	Remained well.
Case 18. Yellow fever. Died in 7 days.	"	407	4th	3	39.8 in 5 days. Trace of icterus.	" "	"	" "

Apparently some of the guinea pigs previously inoculated with the blood from yellow fever cases were refractory to the subsequent inoculation with a known virulent organ emulsion. As shown in Table I, those which resisted the infection were found to have had a febrile reaction of varying degree and duration on about the 5th day after the injection of the blood. This seemingly unimportant fever reaction must have been the result of a very mild abortive infection totally unsuspected on account of the absence of the main symptom, the jaundice. This fact is proved, in my opinion, by the acquired resistance of an animal which, when a sufficient quantity of a passage strain of *Leptospira icteroides* is introduced, is so susceptible. There is reason to believe that the strains of *Leptospira icteroides* as they occur in man are on the whole less virulent to the guinea pig and are incapable of producing a fatal infection from the beginning except in rare instances. It may be recalled here that with one strain (Case 2) the organism did not become thoroughly adapted to the guinea pig until it had been passed through three generations in this animal.[1]

A complete, or nearly complete refractoriness or resistance to *Leptospira icteroides* was demonstrated in sixteen guinea pigs previously inoculated with the blood of yellow fever patients, representing nine out of fourteen cases of yellow fever. Four out of six guinea pigs injected with the blood of two other yellow fever patients died, and two survived. In this group the protection was present in some of the guinea pigs only. In a doubtful case one of the two guinea pigs resisted a subsequent infection. On the other hand, of six guinea pigs, which received the blood of three patients, all died with the typical symptoms and lesions when tested later with a virulent passage strain of *Leptospira icteroides*. Of ten, which received injections of blood from four malarial patients, all proved to be susceptible to a later inoculation with the same strain of *Leptospira icteroides* that was used in the foregoing experiments.

Of the guinea pigs representing the yellow fever group, those which had had a febrile reaction several days after the injection of the blood are the ones which acquired the immunity against the inoculation of *Leptospira icteroides*. There seems to exist some relation between the febrile reaction and the acquired immunity. In several instances, moreover, these guinea pigs showed a suspicious trace of

jaundice in the scleras some time after the height of fever had passed, but the disease had failed to advance further. If the animals had been killed at the proper time and transfers made to normal guinea pigs the virulence of the causative organism might have been gradually increased to reproduce the disease more completely in later passages. As a matter of fact it was possible to accomplish this in one instance (Case 2).

The injection of blood from some cases of typical yellow fever into guinea pigs caused a rise of temperature in about 5 days similar to that of other cases, and there was even a suspicion of a trace of icterus in the scleras, yet no protection against a later infection with a passage strain of *Leptospira icteroides* (Case 1) could be demonstrated. Perhaps this lack of protection may be explained by assuming the strain variations among different strains of the leptospira.

Another point of interest brought out in this series is that the injection of organ emulsions obtained from fresh postmortem material (Case 27) conferred upon guinea pigs sufficient protection against a subsequent infection to prevent its being fatal, although these animals showed fever and jaundice. It is possible that the emulsion contained a certain amount of the antigen and produced a mild immunity, or a limited amount of the immune bodies.

SUMMARY.

The majority of guinea pigs inoculated with the blood of yellow fever patients escaped a fatal infection.

There were a number of instances in which the inoculation of yellow fever blood induced in these animals a temporary febrile reaction on the 4th or 5th day, followed in some cases by slight jaundice, but with a rapid return to normal. Most of these guinea pigs when later inoculated with an organ emulsion of a passage strain of *Leptospira icteroides* resisted the infection. On the other hand, the animals which had previously been inoculated with the blood of malaria patients or normal guinea pigs died of the typical experimental infection after being inoculated with the infectious organ emulsion.

It appears from the results just described that a number of non-fatal, mild, or abortive infections follow the inoculation of blood of yellow fever patients into guinea pigs. The fact that such animals manifested refractoriness to a subsequent attempt to infect with a highly virulent passage strain of *Leptospira icteroides* is an indication, judging from the reciprocal immunity reaction, that they actually passed through an infection with the same organism, or a strain closely related to it, as that which was used for the second infection experiment

ETIOLOGY OF YELLOW FEVER.

V. Properties of Blood Serum of Yellow Fever Patients in Relation to Leptospira icteroides.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 2, 1919.)

Following the isolation of *Leptospira icteroides* from a case of yellow fever a series of experiments was instituted with a view to establishing the relation between the organism and the disease. As stated elsewhere,[1] the organism reproduces in experimental animals all the symptoms and lesions observed in man. From the standpoint of immunity the question arose whether or not the serum of yellow fever convalescents would have a specific influence upon the organism.

Blood was drawn from the median basilic vein of the patient and the serum mixed with *Leptospira icteroides* in the form of organ emulsion from infected guinea pigs or culture and injected into the peritoneal cavity of a normal guinea pig. After a period of 30 minutes to 1 hour the fluid was drawn from the peritoneal cavity by means of a sterile capillary pipette and examined under the dark-field microscope (Pfeiffer's phenomenon). The animals were allowed to live until the results of the inoculations were evident. As Table I shows, in some of the experiments, in which the amount of the infectious emulsion or culture used was too large, no certain protection of the animal from the final fatal infection was obtained with the serum of convalescents, notwithstanding the fact that such serum produced a definite Pfeiffer phenomenon in the peritoneal cavity. When a smaller quantity of the infecting material was used in combination with convalescent serum most of the animals were saved, while the controls with serum from patients not suffering from

[1] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 585.

TABLE I.

Effect of Serum from Yellow Fever Patients upon Leptospira icteroides.

Case No.	Sex.	Age.	Severity of infection.	Day on which serum was taken.	Amount of serum.	*Leptospira icteroides.* Strain 1.		Pfeiffer phenomenon.	Result.
						Liver emulsion.	Culture.		
		yrs.			*cc.*	*cc.*	*cc.*		
9	M.	19	Mild. Recovered.	2nd	1	0.2		−	Died in 8 days.
9	"	19	" "	10th	1	2		+	Survived.
2	"	23	" "	12th	2	2		+	"
14	"	23	Severe. "	10th	2	2		<+	Died in 7 days.
Control.	"	21	Malaria.		2	2		−	" " 7 "
"	"	20	"		2	2		−	" " 7 "
"					Saline, 1 cc.	2		−	" " 7 "
"					Saline, 2 cc.	2		−	" " 6 "
7	M.	21	Severe. Recovered.	16th	1	0.1		+	Survived.
12	F.	40	" "	11th	2	0.1		+	"
9	M.		Diagnosis (?). Recovered.	30th	2	0.1		−	Died in 6 days.
Control.					Ringer, 2 cc.	0.1		−	" " 10 "
"					Ringer, 2 cc.	0.1		−	" " 9 "
16	M.	25	Severe. Recovered.	19th	1		1	<+	All these sera
26	"	21	" "	18th	2		1	−	were separated from
29	F.	34	Mild. "	13th	1		1	−	the clot in 48
19	M.	25	Severe. "	24th	2		1	+	hrs. and then
30	"	21	Mild. "	14th	2		1	+	kept on ice for
31	"	22	Moderate. "	20th	1		1	+	2 to 4 wks.
23	"	16	" "	22nd	2		1	+	when they
32	"	20	Mild. "	15th	2		1	+	were tested.
33	"	11	" "	7th	2		1	+	The negative
8	"	21	Severe. "	14th	2		1	+	results with
20	"	20	Moderate. " (also malaria).	13th	2		1	<+	the sera of Cases 26 and
34	F.	30	Mild. Recovered.	12th	2		1	+	29 may be due to deterioration through age.

yellow fever, and with saline solution died with the typical symptoms.

The number of cases studied (eighteen) was limited, owing to the pressure of more urgent problems under investigation at the same time. It seems, however, to have been sufficient to establish the specific reaction which exists between *Leptospira icteroides* and the serum of yellow fever convalescents (fifteen positive, 83 per cent). In the case of one patient the serum did not have any effect upon the organism when tested on the 2nd day of the illness but was protective on the 10th day.

Sera from malarial patients in no case showed any action antagonistic to *Leptospira icteroides*. The malarial patients were all mountaineers and consequently non-immune to yellow fever. The negative results obtained with sera derived from doubtful cases of yellow fever, all so mild as to make it difficult to recognize the disease, might have been due to the absence of antibodies, or, if there were any, to the fact that they were too weak to produce a definite reaction under the experimental conditions, or, a not impossible assumption, to the existence of a variation among many strains.

As will be described later, ten normal sera from healthy, non-immune soldiers[2] were also examined before the vaccination of the latter with killed cultures of *Leptospira icteroides*, but none of them had any effect upon the organism.

SUMMARY.

The serum from a number of persons recovering from yellow fever in Guayaquil was studied with a view to establishing its possible immunological relationship with a strain of *Leptospira icteroides* derived from one of the yellow fever patients. For this purpose the serum of convalescents was mixed either with an organ emulsion of a passage strain, or with a culture of the organism, and inoculated intraperitoneally into guinea pigs.

[2] I am indebted for these specimens to Dr. Carlos A. Miño, Assistant Director of the Department of Health at Quito, to Dr. E. Salgado V., also of the Department of Health, and to Dr. Víctor M. Bayas, Surgeon of the "Bolivar" Regiment.

The Pfeiffer reaction was first studied, and then the animals were allowed to live until the controls, inoculated with the same emulsion or culture of *Leptospira icteroides* but without the serum, or with serum from patients suffering from other diseases than yellow fever, had died of the experimental infection with typical symptoms A positive Pfeiffer phenomenon was observed in fifteen of the eighteen convalescent cases studied, or approximately 83 per cent. Sera from ten non-immune soldiers and from two malaria patients gave uniformly negative results. Protection from an ultimate fatal infection was afforded some of the guinea pigs which received the serum of yellow fever convalescents, while the control animals succumbed to the infection with typical symptoms. In one instance, in which the serum was tested on the 2nd and the 10th days of disease, a Pfeiffer reaction was demonstrated, as well as protective property against the infection, in the specimen from the 10th but not in that from the 2nd day.

From the foregoing observations of immunity reactions it appears highly probable that *Leptospira icteroides* is etiologically related to yellow fever.

ETIOLOGY OF YELLOW FEVER.

VI. Cultivation, Morphology, Virulence, and Biological Properties of Leptospira icteroides.

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Plates 1 to 3.

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Cultivation.

As the nature of the causative agent of yellow fever was unknown, it was necessary at the beginning of these experiments to formulate a special method of cultivation. The methods employed have been similar to those recommended[1] for the cultivation of *Leptospira icterohæmorrhagiæ* (Inada and Ido). Instead of the serum and citrate plasma of the rabbit or other animal, serum and plasma from non-immune persons were used during the early stage of the cultivation experiments. The principal medium consisted of a mixture of 1 part of the serum and 3 parts of Ringer solution, used in a combination of the liquid form and a form made semisolid by adding melted neutral agar (0.3 per cent), the liquid half (8 cc.) of the medium being superimposed on the semisolid half (8 cc.) in a tall culture tube such as that used in the cultivation of spirochetes.[2]

The first step in the inoculation of the medium was to mix about 0.5 to 1 cc. of the citrate blood, drawn from the median basilic vein of the patient, with the lower or semisolid portion of the medium, while the latter was still in the fluid state (42°C.), and allow the mixture to solidify by cooling. The serum-Ringer dilution was then poured on the semisolid portion and about 0.5 to 1 cc. more of the same blood introduced. A layer of paraffin oil was finally added to

[1] Noguchi, H., *J. Exp. Med.*, 1918, xxvii, 575.

[2] Noguchi, H., *J. Exp. Med.*, 1912, xvi, 199.

cover the surface of the medium. When making a culture with the citrate blood from a patient no human citrate plasma was added to the liquid portion of the medium, as the plasma contained in the blood was sufficient to form a loose fibrin throughout that portion. When subsequent subcultures were set up, however, 0.5 to 1 cc. of citrate human or rabbit plasma was introduced into the liquid portion after inoculation. The presence of a loose cobweb fibrin in the culture medium seems to favor the growth of certain organisms.[1] The conditions provided for in this form of culture medium would allow the growth of various microorganisms requiring different degrees of oxygen tension. In a later period 2 to 3 cc. of the citrate blood were used for each of two or three large flasks (50 cc.) with correspondingly larger quantities of culture medium (25 cc.).

Direct Cultivation from Yellow Fever Patients.

Cultures were made from eleven cases of yellow fever, with only three successful isolations of the leptospira. In the first instance (Case 1) I failed to detect the organisms under the dark-field microscope, but in a culture 3 days old (kept at 26°C.) a few active leptospiras were seen. This was inoculated into four guinea pigs, all of whom died later of the typical experimental infection. One of the guinea pigs had epistaxis and melena in addition to intense jaundice, advanced degeneration of the liver, acute parenchymatous nephritis, ecchymoses in the lungs, stomach, and intestines.[3] This culture was presently lost through a secondary fungus contamination, which was difficult to avoid under the conditions in which the work had to be carried on.

The second positive growth was obtained with the blood derived from Case 4 on the 3rd day of the disease. The organisms were readily detected in the culture after 5 days at 30°C. A few leptospiras were present in the blood when carefully examined in the stained preparations, and the guinea pigs inoculated with this specimen came down with the typical symptoms in 8 days. The culture proved to be pathogenic for guinea pigs.

[3] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 565.

The third successful direct cultivation of the organism from yellow fever patients was obtained with the blood from a fatal case (Case 6). The blood was drawn on the 5th day of the disease and put immediately into six tubes containing the culture medium (October 19, 1918). On October 26 one of the inoculated tubes showed the presence of the leptospira under the dark-field microscope. The culture proved to be capable of producing the typical symptoms and lesions in guinea pigs, pups, and marmosets.[4]

Cultivation from Experimental Animals.

The method employed for obtaining a culture of *Leptospira icteroides* was the same as that outlined for direct cultivation from human blood, except that normal rabbit serum and citrate plasma were used instead of human. The blood was obtained from the heart before the death of the animal. The method was not always successful in the first generation, but it was nevertheless the most reliable of the various combinations tried. In a later subculture the addition of the citrate plasma becomes unessential, although a better growth is had when it is added.

Six strains (Cases 1 to 6) of yellow fever leptospira have been maintained to date by passage in guinea pigs. No direct culture was obtained with the blood from Cases 2, 3, and 5, although cultures were finally obtained from the blood of guinea pigs or marmosets inoculated with the passage strain. These cultures, whether obtained directly from the blood of yellow fever patients or indirectly by way of animal inoculation, were found to be uniform in their characteristics and could be maintained in culture for many months. The leptospira isolated from yellow fever cases is extremely sensitive to any alien microbic intrusion, not surviving the slightest contamination in culture, and the failure to obtain a culture directly from yellow fever cases can in every instance be explained through the occurrence of secondary contamination.

When the guinea pigs reach the stage of collapse, with intense jaundice, it is seldom possible to detect the leptospira in the circulation, and a positive transfer to normal guinea pigs becomes uncertain.

[4] Noguchi, H., *J. Exp. Med.*, 1919, xxix, 585.

Cultures set up with this blood usually remain sterile, and the leptospira is not found in the liver and kidneys. In experimental infectious jaundice, on the contrary, the leptospira was almost always found in the later stage of the infection. The extreme lability of *Leptospira icteroides* may account for the negative animal inoculation and microscopic findings in so many cases of yellow fever.

Morphology.

The organism which occurs in the blood and tissues in yellow fever patients in Guayaquil, as well as in those of animals experimentally infected with the blood or tissue of yellow fever patients, is an extremely delicate filament measuring about 4 to 9 microns in length and 0.2 of a micron in width along the middle portion. It tapers gradually toward the extremities, which end in immeasurably thin sharp points. The entire filament is not smooth but is minutely wound at short and regular intervals, the length of each section measuring about 0.25 of a micron. The windings are so placed as to form a zigzag line by the alternate change of direction of each consecutive portion at an angle of 90°.

The organism is unrecognizable by translucent light but becomes quite visible under a properly adjusted dark-field illumination. It possesses an active motility, consisting in vibration, rotation, rapid bipolar progression, and sometimes twisting of parts of the filament. When it encounters a semisolid substance it penetrates the latter by a boring motion, and while passing through it the body assumes a serpentine aspect with few undulations, the elementary windings undergoing no modification.

The organism manifests remarkable flexibility to almost any angle while changing its course of progression in a semisolid medium. In a fluid medium it has fewer and quite characteristic movements. One end is usually bent in the form of a graceful hook, and, while rapidly rotating, the organism proceeds in the direction of the straight end, the hooked end apparently serving as a sort of rear propeller. When extricating itself from an entanglement, however, the same hooked end seems to act like the front propeller of an airplane. Many specimens are seen with both ends hooked, the organism then ro-

tating in a stationary position unless one hook is larger and more powerful as a propeller than the other. The rapid rotation makes the organism appear like a chain of minute dots. From the dynamic point of view the portions which include the several windings from the extremities represent the motor apparatus of the organism. I have never seen a specimen that doubled at the middle portion of the body while lying in a free liquid medium. The motor or terminal portions may be regarded as comparable with the flagella or terminal filaments seen in a spironema or treponema.

The organism is difficult to stain with ordinary aniline dyes, but can be made distinct by osmic acid fixation and one of the Romanowsky stains (Giemsa, Wright, Leishman). When stained with Fontana or carbolized gentian violet solution after mordanting with 5 per cent tannin plus 1 per cent phenol the organism appears as a moderately heavy, slightly undulated filament without a clear elementary indentation. The peculiar forms resembling the letters C and S are quite characteristic. Specimens fixed with methyl alcohol seldom retain the elementary spirals. The beauty of the organism as it appears by dark-field illumination is never well retained in a stained preparation, even in the best specimens. In the latter it appears almost as a totally different organism.

From the findings described it is evident that the present organism falls in the general order of so called spirochetes, but in the strict sense of the term it is neither a bacterium, a spirochete, a spironema, nor a treponema, but belongs to the genus *Leptospira*, of which *Leptospira icterohæmorrhagiæ*, *Leptospira hebdomadis*,[5] and *Leptospira biflexa* have already been described.[1]

The study of the strains of *Leptospira icteroides* obtained from yellow fever cases in Guayaquil showed the organisms to be of somewhat smaller dimensions than the various strains of *Leptospira icterohæmorrhagiæ* in my possession (six strains), as is readily seen from the photographs of the organisms shown in Figs. 1 to 9. The difference is striking when the pictures of the two organisms are compared, particularly in the case of Strain 6 of *Leptospira icteroides*, which is considerably smaller than any of the other strains.

[5] Ido, Y., Ito, H., and Wani, H., *J. Exp. Med.*, 1918, xxviii, 435.

The strains of *Leptospira icterohæmorrhagiæ* isolated from wild rats caught in Guayaquil are also shown (Figs. 10 to 13). These are seen to be similar to the other strains of *Leptospira icterohæmorrhagiæ*, shown in Figs. 5 to 9, and are coarser than the organism obtained from yellow fever cases.

The photographs shown in the three plates were taken for the purpose of comparison at the same time, under similar conditions, and with the same magnification.

Cultural Properties.

Leptospira icteroides does not multiply in a medium in which there is no access to oxygen. In a dense solid medium it grows well within the zone or layer to which a trace of oxygen can still penetrate, but no deeper. It grows best when the supply of oxygen is not excessive, as when a thin layer of liquid paraffin is poured over the surface of the culture medium. A certain amount (above 10 per cent) of a suitable blood serum is essential for its growth. Various bacterial culture substances such as peptone, meat extract, various carbohydrates in different forms, or combinations, are unsuitable; their presence in the serum-containing media apparently neither favors nor impedes development. The percentage of sodium chloride (tried as high as 2 per cent) in the medium seems to have but little influence, and either isotonic saline, Ringer solution, or distilled water may be used as diluent. The organism is highly sensitive to the reaction of the medium, the optimum growth being obtained with a reaction slightly alkaline to litmus paper, not stronger than 0.025 N. It grows well in a neutral medium, but not in one with an acid reaction to litmus paper.

The addition of phenol red to culture media in a ratio of 1 cc. of a 0.0025 per cent solution to 10 cc. of medium has no perceptible disturbing effect upon the growth of *Leptospira icteroides*. Growth takes place in culture tubes in which phenol red indicates the values ranging from pH 6 to pH 7.4. In the case of cultures containing rabbit serum phenol red is gradually decolorized to a trace of pink.

Growth is much more rapid at a temperature of 37°C. than at 25–26°C., but the organisms remain viable much longer at the latter

temperature. No growth is obtainable at a temperature above 42°C. or below 10°C.

Erythrocytes present in the culture do not undergo any special alteration that can be ascribed to the growth of this organism, nor does the hemoglobin. The serum proteins seem in no wise modified and remain transparent. No external changes of the culture media, except a light layer of grayish haze over the surface of a solid or semisolid medium observed in richly growing old cultures, take place, and for this reason the growth of the organism can be ascertained microscopically only.

It has been noticed that *Leptospira icteroides* shows a particular preference for a semisolid medium such as is provided by the presence of agar (0.3 per cent) or by loose fibrin. They entangle themselves in the substance in large numbers and move about in it very actively. Continuous multiplication goes on in this type of medium. After a few weeks the growth may become so dense as to render the uppermost layer of the medium faintly grayish. This peculiarity of the organism may partly account for its predilection for the parenchymatous organs such as the liver and kidney.

Leptospira icteroides multiplies through transverse fission.

Virulence.

While the pathogenic properties of *Leptospira icteroides* for different species of animals have not been exhaustively studied, it has been shown that most of the domestic animals, such as the donkey, horse, sheep, pig, and cat are completely refractory to the injection of the organism. Very young dogs, not older than 6 or 7 weeks, are found to succumb to experimental infection. None of the birds so far employed for experiment has been found to be susceptible.[3] Among the mammals the guinea pig appears to be most susceptible and the marmoset somewhat less so. For this reason the guinea pig has been chosen for determining the degree of virulence of several strains of *Leptospira icteroides*.

The mode of inoculation consisted in intraperitoneal injection in descending doses of a culture 2 to 3 weeks old. The guinea pigs used varied from 300 to 350 gm. A 2 to 3 weeks old culture (26°C.) of this organism, grown in a semisolid rabbit serum medium with

0.15 per cent agar, may contain 50 to 100 leptospiras per field (Leitz $\frac{1}{12}$ oil immersion and ocular 4). In order to arrive at an accurate determination of virulence different strains would have to be used in correspondingly comparable concentrations. But this is extremely difficult in the case of an organism which forms entangled masses of many individuals or shows a tendency to congregate in varying numbers about the particles of culture medium. In the present series of experiments suspensions of cultures of different strains were so prepared as to make each contain approximately an equal number of organisms in the suspension from which higher dilutions were prepared. Each strain was used in successive tenfold dilutions, and one or two guinea pigs were inoculated with 1 cc. each of each dilution. Because

TABLE I.

Determination of Virulence of Leptospira icteroides, Strain 1.

Guinea pig No.	Quantity of culture.	Incubation, or time after inoculation to onset of fever.	Result.
	cc.	*days*	
1	1	3	Died in 7 days.
2	0.1	4	Survived (!). No jaundice.
3	0.01	5	Died in 9 days.
4	0.001	$3\frac{1}{2}$	" " 8 "
5	0.0001	4	" " 9 "
6	0.00001	5	" " 10 "
7	0.000001	No fever.	Survived.

of individual variations in resistance among the guinea pigs in all later experiments two animals were used for each dilution.

Four strains of *Leptospira icteroides* were studied in this way. The results are recorded in the following protocols.

Experiment 1.—Aug. 10, 1918 (at the Guayaquil Yellow Fever Hospital) Strain 1. 18 day culture of the second generation, grown on semisolid human serum agar at 30°C. (Table I.)

Experiment 2.—Dec. 2, 1918. Strain 3. 20 day culture of the third generation, grown on semisolid rabbit serum agar medium at 26°C. (Table II.)

Experiment 3.—Jan. 2, 1919. Strain 5. 3 week culture of the third generation, isolated from a marmoset experimentally infected with a visceral emulsion from a guinea pig which died of the typical infection after inoculation with blood from a fatal yellow fever case. The culture was grown on the same medium as that used for Strain 6, at 26°C. (Table III.)

TABLE II.

Determination of Virulence of Leptospira icteroides, Strain 3.

Guinea pig No.	Quantity of culture.	Incubation, or time after inoculation to onset of fever.	Result.
	cc.	*days*	
1	1	$3\frac{1}{2}$	Died in 8 days.
2	1	5	Survived (!).
3	0.1	4	Died in 9 days.
4	0.1	5	" " $8\frac{1}{2}$ "
5	0.01	5	Survived (!).
6	0.01	$5\frac{1}{2}$	Died in 11 days.
7	0.001	4	" " 9 "
8	0.001	5	Survived.
9	0.0001	5	"
10	0.0001	No fever.	"
11	0.00001	7	"
12	0.00001	No fever.	"
13	0.000001	" "	"

TABLE III.

Determination of Virulence of Leptospira icteroides, Strain 5.

Guinea pig No.	Quantity of culture.	Incubation, or time after inoculation to onset of fever.	Result.
	cc.	*days*	
1	1	3	Died in 7 days.
2	0.1	3	" " 8 "
3	0.1	$2\frac{1}{2}$	" " 6 "
4	0.01	$3\frac{1}{2}$	" " 7 "
5	0.01	$4\frac{1}{2}$	" " 9 "
6	0.001	5	" " 10 "
7	0.001	$3\frac{1}{2}$	" " 8 "
8	0.0001	5	" " 10 "
9	0.0001	4	" " 9 "
10	0.00001	6	Survived.
11	0.00001	No fever.	"
12	0.000001	" "	"
13	0.000001	" "	"

Experiment 4.—Jan. 2, 1919. Strain 6. 20 day culture of the third generation, directly derived from human blood (not passed through guinea pigs), grown on semisolid rabbit serum agar at 26°C. There were about 25 organisms per field in the original suspension. (Table IV.)

The foregoing experiments show that the strains of *Leptospira icteroides* possess, on the whole, a strong virulence for guinea pigs. In two cases (Nos. 1 and 6) the minimal lethal dose was 0.00001 cc., in one (No. 5) 0.0001 cc., and in another (No. 3) 0.001 cc. But in the experiments with Strains 1 and 3 some of the guinea pigs receiving as large a quantity as 1 cc. or 0.1 cc., showed only a transient febrile reaction and speedily returned to normal, notwithstanding the fact that 0.0001 part of these doses killed other guinea pigs in the same series of experiments. This is not altogether exceptional,

TABLE IV.

Determination of Virulence of Leptospira icteroides, Strain 6.

Guinea pig No.	Quantity of culture.	Incubation, or time after inoculation to onset of fever.	Result.
	cc.	*days*	
1	1	4	Died in 7 days.
2	0.1	3	" " 10 "
3	0.1	3½	" " 6 "
4	0.01	4	" " 8 "
5	0.01	5 (?)	" " 7 "
6	0.001	3	" " 9½ "
7	0.001	5	" " 10 "
8	0.0001	4	" " 10 "
9	0.0001	Doubtful.	Survived.
10	0.00001	3	Died in 8 days.
11	0.00001	6	Survived.
12	0.000001	4	"
13	0.000001	No fever.	"

because in the higher dilutions there were instances in which a smaller dose induced a fatal infection while a larger one failed to do so. Again, in fatal instances the severity of the infection did not parallel the amount of culture injected. In other words, the susceptibility of guinea pigs varies considerably among different individuals. In another series of experiments, not yet reported, it was noticed that certain guinea pigs possess an almost complete natural immunity to *Leptospira icteroides*. This becomes an important factor in a consideration of the percentage of successful transmissions of this organism from human cases to guinea pigs.

Gradual Loss of Virulence through Cultivation.

All the strains of *Leptospira icteroides* were brought to New York from Guayaquil on semisolid rabbit serum agar. The cultures were kept at ordinary temperature during the journey (about 28°C. in the tropics and 15°C. after reaching the United States). They had been renewed in Guayaquil on October 26, 1918, and were tested for their pathogenicity for guinea pigs in New York on December 2, 1918; that is, 37 days after the transfer into new media. Intraperitoneal inoculations were made into guinea pigs of 1 cc. of Strains 1, 3, 4, 5, and 6, and the animals developed the usual symptoms and lesions characteristic of the infection produced by these strains, showing that under the circumstances described the organism remained virulent for 37 days.

On December 9, 1918, some of the older cultures of Strains 1 and 3, which had stood over 4 months since cultivation, were also tested, with varying success. Strain 1 was still quite virulent, but Strain 3 failed to produce a fatal infection. In a subsequent experiment, however, by using six guinea pigs, each being inoculated with 2 cc. of the culture intraperitoneally, it was possible to obtain a fatal infection in one of the animals. Through this guinea pig the virulence of the culture was again raised to its original height; that is, it again became capable of causing typical infection in guinea pigs in smaller quantities.

It should be noted that examination of the viscera, especially the lungs, of the guinea pigs which escaped death or severe infection from the inoculation of an attenuated strain, by killing them at the end of about 14 days from the time of inoculation, usually revealed the presence of old hemorrhages of greater or less extent in the lungs. Perhaps it may prove a useful procedure for ascertaining the outcome of transmission to inoculate several guinea pigs with the blood of a yellow fever patient and examine the lungs within a period of from 10 to 14 days. In this way, notwithstanding the absence of striking external manifestations, the results of inoculation can be more accurately followed. Unfortunately this fact was not known at the time of the experiments, reliance being placed upon the development of a fatal infection.

For the past 4 months horse serum has been used for culture media, since it was easily obtainable at much less expense and in larger quantities than rabbit or sheep serum. It is far less satisfactory, however, for the cultivation of *Leptospira icteroides*, than sheep serum, which in turn is much inferior to rabbit serum. In testing out the virulence of different strains of the organism recently, rather rapid loss of virulence has been encountered. The reason was not at first clear, the period of time being comparatively short, but it soon became evident that cultivation of *Leptospira icteroides* on sheep or horse serum media leads to rapid diminution of virulence, since the cultures grown on rabbit serum media remained pathogenic. By following the course of development of these strains in media containing rabbit serum and phenol red it was found that the original pinkish color of the indicator gradually becomes paler until it fades to a trace. The color may be brought back to a deeper hue by the addition of disodium phosphates, but it never reaches the original grade. Apparently a change takes place in the media as well as in the indicator through the growth of the organism. Such a change has not been observed in the cultures grown on media containing sheep or horse serum. Whether the persistence of virulence of the organism in the media containing the rabbit serum has any relation to the phenomenon just described or whether they are two unrelated coincidental phenomena has not been further studied.

Resistance and Viability.

Leptospira icteroides is a non-spore-bearing organism and offers little resistance to the action of heat, desiccation, putrefaction, or disinfectants.

Heating to 55°C. for 10 minutes or freezing and thawing kill the organism, and complete desiccation promptly destroys its vitality.

In the presence of various bacteria, such as *Bacillus coli*, *Bacillus aerogenes*, *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus pyocyaneus*, pneumococcus, staphylococcus, *Streptococcus hæmolyticus*, etc., *Leptospira icteroides* is destroyed within a short time. The more numerous the bacteria the quicker the disappearance of the leptospira; hence in decomposing excreta or urine, sewer or stagnant water, or in con-

taminated foodstuffs, no leptospira can be found 24 hours after being introduced into them.

On the other hand, certain contaminating fungi or non-putrefactive and non-acid-producing bacteria, sometimes bacilli and sometimes cocci, have been found growing in the cultures of *Leptospira icteroides* without seriously interfering with the viability of the latter, which are actively motile among the intruding fungi or bacteria. In such a contaminated culture these intruders do not cause any perceptible modification of the culture medium, except that their discrete colonies may be found imbedded here and there in the medium.

When a pure culture of the organism was poured into a cup of sterile distilled water and left unprotected from the air or dust the leptospiras survived several days, but finally disappeared, partly because of the lack of nutrition and partly because of bacterial growth. The leptospira intentionally added in large quantity to fecal matter kept at room temperature disappeared within a few hours.

On several occasions attempts were made to infect the larvæ of *Stegomyia calopus* by introducing emulsions of liver or kidney containing a large number of the organisms into the receptacle with the larvæ, but no leptospira could be found in such a mixture after 2 hours. In this respect the virus of yellow fever is one of the least resistant of all pathogenic organisms which have been obtained in culture. In my experience there has seldom been an impure culture of this organism.

Another interesting phenomenon in connection with the organism in question is that it soon dies out; it may degenerate within 12 hours in a piece of liver or kidney removed from an infected guinea pig and kept at a temperature of about 10°C. In hundreds of instances a leptospira was found only rarely in the liver, kidney, or blood from guinea pigs which had died of typical experimental yellow fever several hours before autopsy. In this respect *Leptospira icteroides* differs considerably from *Leptospira icterohæmorrhagiæ* isolated from the cases of infectious jaundice in Japan or Europe, the latter being still easily recoverable from animals kept over night after death.

With regard to the resistance of the organism to the action of various ordinary disinfectants the work is still incomplete. It has been found, however, that it is readily killed within 5 minutes by 2 per

cent phenol or 0.1 per cent bichloride of mercury. In a 10 per cent solution of sodium taurocholate, sodium glycocholate, or sodium cholate the organism promptly disintegrates, but saponin has no injurious effect upon it. Human or animal bile dissolves the organism rapidly when used in concentrations stronger than 30 per cent.

Filterability.

A noteworthy characteristic of *Leptospira icteroides* is its ability to pass through the pores of filters. Some experiments to determine this point were carried out as early in the investigation as the transmission of Strain 1 from the human case to guinea pigs. By the use of Berkefeld filters V and N with suction by means of a water pump it was possible to filter an emulsion of the liver and kidney of a guinea pig experimentally infected with the passage strain 7 days previously. The clear filtrates, which were bacteriologically sterile, were inoculated intraperitoneally into normal guinea pigs in doses of 10 cc. each on August 8, 1918. Both animals came down with typical symptoms after $7\frac{1}{2}$ and 8 days respectively. In the blood of these animals a small number of leptospiras were demonstrated 24 hours before death. In the emulsions of the liver and kidney the organisms were also present, and upon further passage to normal guinea pigs the emulsion proved to be infectious.

Possibility of the Existence of a Granular Phase in the Life of Leptospira icteroides.

That there may exist a granular phase of life in various members of the family of spirochetes has been repeatedly suggested by investigators. Balfour, Fantham, Leishman, and Todd[6] advanced the idea that the spirochetes of relapsing fevers in man and fowls pass through a granular stage at some time in their life. The following observation seems strongly to suggest the possibility that this phenomenon also occurs in the life of *Leptospira icteroides*.

[6] Balfour, A., *Internat. Congr. Med.*, 1913, xxi, 275. Fantham, H. B., *Ann. Trop. Med. and Parasit.*, 1914, viii, 471. Leishman, W. B., *Internat. Congr. Med.*, 1913, xxi, 282. Todd, J. L., personal communication.

The various cultures of *Leptospira icteroides* made at Guayaquil on October 26, 1918, were brought back to New York on November 24 without special accident. But on examination no leptospira could be found in any of the tubes containing cultures of Strain 5, although the other cultures were growing well. A thorough examination of the eleven tubes of Strain 5 was continued for several days without success.

Six culture tubes which were made on October 18, 1918, with the blood from Marmoset 4, severely infected with Strain 5, and which had been showing a fairly good growth on October 26, were also examined. These tubes showed no spiral organisms. There were large numbers of refringent granules imbedded in the culture medium in which the leptospiras had been abundantly present a month previously. These granules appeared to be the degenerated remains of the leptospiras. The hope of recovering the strain from these cultures was almost abandoned, but as a last resort a dozen guinea pigs were inoculated with 1 cc. of the contents of each of these tubes. Some of these animals in due time came down with typical symptoms. The spiral forms of the leptospiras were found in varying numbers in the blood, liver, and kidneys of these animals, and a culture of the strain was regained.

It is of course possible that these old culture tubes contained the spiral leptospira in such small numbers that they escaped microscopic detection, but it is also possible that they existed in a granular phase under certain conditions.

SUMMARY.

By the employment of methods designed to promote the growth both of aerobic and anaerobic organisms, particularly those belonging to the class of spirochetes, it was possible to obtain a pure culture of a delicate organism, the morphological features of which place it in the genus *Leptospira*. On three occasions, that is, from three out of eleven cases of yellow fever, the organism was directly cultivated. These three strains were found to induce the characteristic symptoms and lesions when tested on guinea pigs. The organism was designated *Leptospira icteroides*.

Leptospira icteroides was also obtained in pure culture from the blood of guinea pigs which succumbed to infection after being inoculated with the blood or organ emulsions from patients suffering from yellow fever. These cultures also proved to be virulent when tested on susceptible animals.

The morphological characteristics and certain biological properties of the organism were considered in detail. It is invisible under translucent illumination and is difficult to stain by most aniline dyes. It is highly sensitive to the presence of bacteria and is rapidly destroyed in a medium in which certain other organisms are present. The presence of blood serum (man, sheep, horse, rabbit, etc.) seems to be essential for its growth. It grows well at a temperature of about 25–26°C. and more quickly at 37°C., though at the latter temperature it dies out within a few weeks. At 25°C. under favorable conditions and in suitable culture media it remains viable for several months without losing its virulence. *Leptospira icteroides* multiplies by transverse division.

The virulence attained by some strains was such that 0.00001 cc. of a culture could induce typical fatal infection in guinea pigs. There exists a considerable variation among guinea pigs in their susceptibility to *Leptospira icteroides*.

The organism is killed within 10 minutes at a temperature of 55°C. and is also destroyed by complete desiccation or freezing and thawing. Bile and bile salts dissolve it in certain concentrations, but not saponin.

Leptospira icteroides passes through the pores of Berkefeld filters V and N, and there is a possibility of its having a granular phase of life under certain conditions.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Dark-field view of a 2 week culture on semisolid rabbit serum agar medium of *Leptospira icteroides*. Strain 6 (Case 6). × 3,000.

FIG. 2. The same. Strain 4 (Case 4).

FIG. 3. The same. Strain 5 (Case 5).

FIG. 4. The same. Strain 3 (Case 3).

PLATE 2.

FIG. 5. Dark-field view of a 2 week culture on semisolid rabbit serum agar medium of *Leptospira icterohæmorrhagiæ*. Japanese strain. × 3,000.

FIG. 6. The same. British strain.

FIG. 7. The same. French strain.

FIG. 8. The same. American Strain 1.

FIG. 9. The same. American Strain 2.

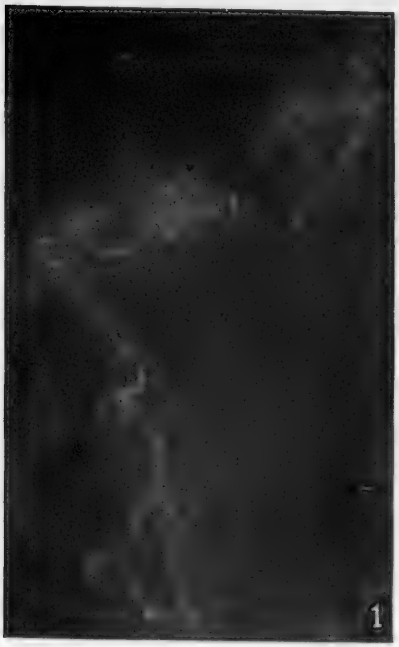
PLATE 3.

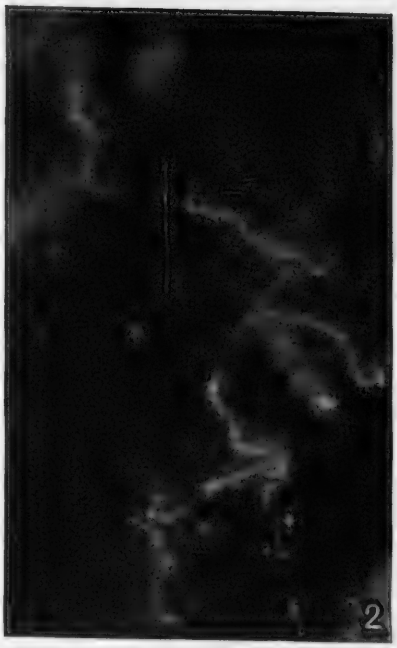
FIG. 10. Dark-field view of a 2 week culture on semisolid rabbit serum agar medium of *Leptospira icterohæmorrhagiæ*. Group 8 strain obtained from wild rats in Guayaquil. × 3,000.

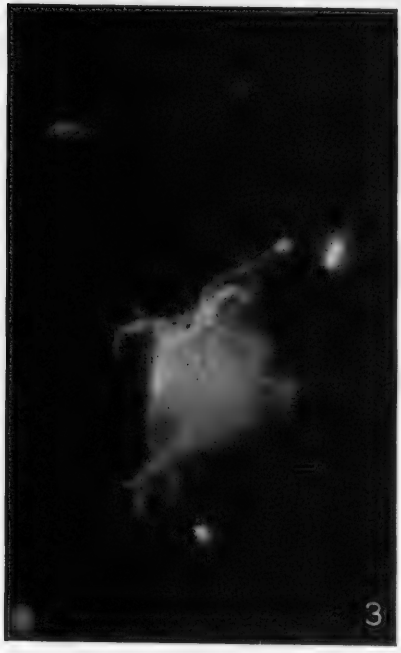
FIG. 11. The same. Group 11 strain.

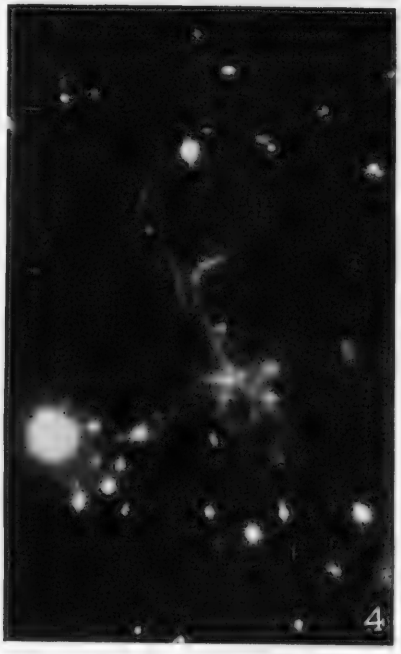
FIG. 12. The same. Group 30 strain.

FIG. 13. The same. Group 30 strain.

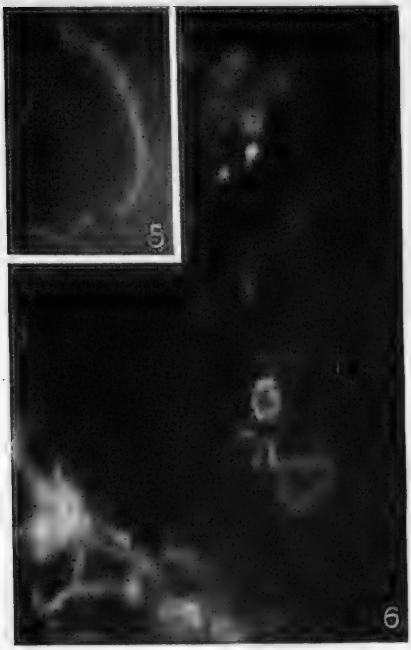


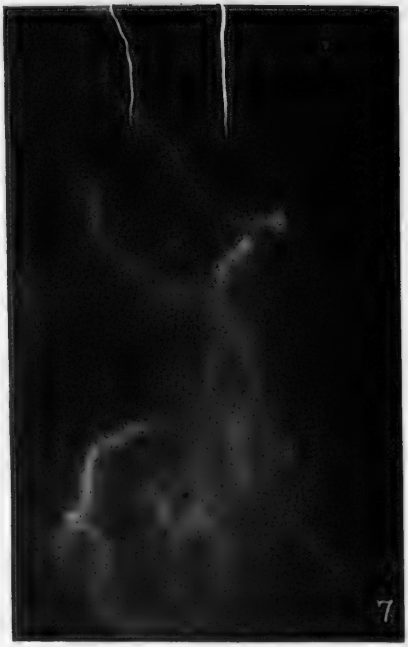






(Noguchi: Etiology of yellow fever. VI.)





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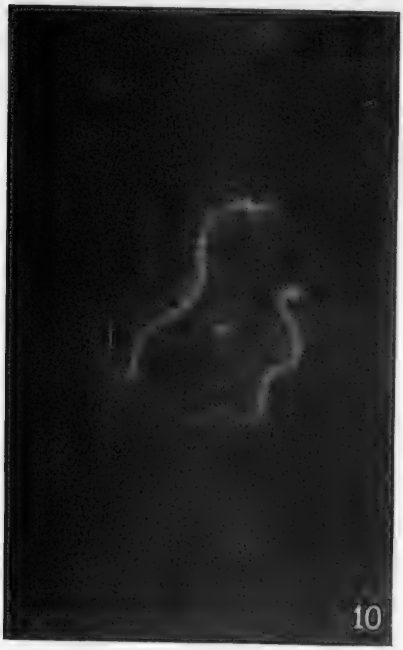

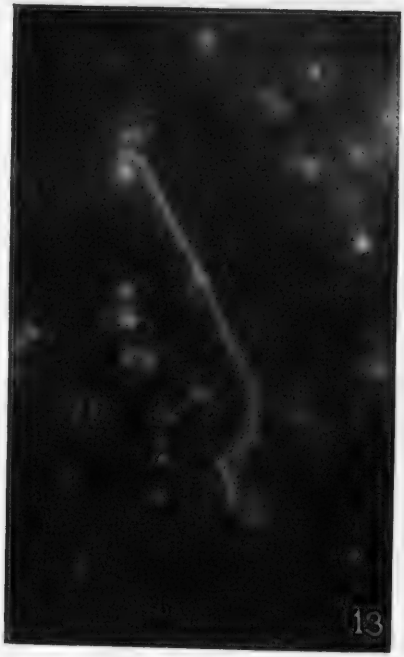

(Noguchi: Etiology of yellow fever. VI.)

GROWTH ACCESSORY SUBSTANCES FOR PATHOGENIC BACTERIA IN ANIMAL TISSUES.

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The subject of vitamines has of late occupied the attention of the biologist and biological chemist. The nature of these substances is still a matter of speculation, but the majority of workers accepts the distinction proposed by McCollum and Davis. These authors recognize two classes of substances, the fat-soluble A and the water-soluble B. A more precise definition has not been possible, because of the failure thus far to isolate and identify the respective compounds. Their presence can be detected only by the effect they produce on the growing organism, usually the white rat. By noting the effect of the addition of various substances to a balanced vitamine-free diet, one can obtain a rough indication of the concentration of food accessory substances in the added material.

The subject of the relation of vitamines to bacteria has recently been approached from two directions. One group of workers studied the bacteria with a view to discovering the source of vitamines. Since plants (Bottomley) and animals (McCollum and Simmonds, Osborne and Mendel, Loeb and Northrop) cannot synthesize these substances, their origin must naturally be looked for among the lower microorganisms. Mockenridge has indeed shown that certain soil bacteria are capable of elaborating these substances, although *Bacillus radicicola*, actively synthetic and of simple food requirements, cannot do so. Pacini and Russell report experiments indicating that the typhoid bacillus grown in Uschinsky's medium produces vitamines. The other group of workers was more concerned in determining the relation of vitamines to the cultivation of the more delicate pathogenic bacteria. Cole and Lloyd demonstrated their importance for the growth of the gonococcus, while Lloyd and Gordon, Hine, and

Flack showed that they were essential also for the growth of the meningococcus. Evidently neither of the organisms can multiply actively in the absence of these substances.

The object of this paper is to report experiments bearing on (*a*) the effect of vitamines on the growth of a number of organisms pathogenic for man; (*b*) the distribution of these substances in animal tissues; and (*c*) the relative significance of the fat-soluble A and water-soluble B in the cultivation of these microorganisms. At present there are few data bearing on these questions in relation to bacteria.

EXPERIMENTAL.

Methods.

In the course of the investigation beef heart, goat blood, rabbit and cat tissues, and human secretions were used. Unless otherwise stated, the method of extraction was always the same. The tissue or organ was obtained as free from blood as possible. The animals were first exsanguinated and the tissues were washed with saline solution, free from visible traces of blood. The material was then weighed, macerated into small bits, suspended in nine times its weight of saline solution, shaken thoroughly, and placed in the ice box over night. The following day the extract was centrifugalized and filtered through a Berkefeld candle. After testing for sterility the extracts were ready for use.

The effect of these extracts was tested by adding graded amounts to nutrient broth or agar, or to phosphate peptone agar and inoculating with small amounts of culture suspensions. The tubes were incubated at 37°C. and observations were made daily for several days. Control tubes without extract were always used.

The following organisms were tested:[1] gonococcus, meningococcus (one para, one regular), Pneumococcus Type I, *Streptococcus hæmolyticus*, *Bacillus diphtheriæ*, *Bacillus pertussis*, *Bacillus influenzæ*.

[1] Cultures of *B. typhosus* and of the Shiga and Flexner varieties of *B. dysenteriæ* were included in the early experiments, but as no differences were observed between the growth on the control media and on those containing the extracts, they were later excluded.

To avoid the error that would result from carrying over vitamines with the bacterial mass, small inocula were used. Usually 0.1 cc. of a 24 hour broth culture was suspended into 5 cc. of peptone or saline solution, and of this suspension 0.1 cc. was used for inoculating broth tubes and a 4 mm. loop for the agar slants. Before making the suspensions the cultures were first diluted to a turbidity corresponding to a 24 hour broth culture of the pneumococcus.

Results.

Most of the experiments reported below were repeated from one to four times. Either broth or agar media or both were used. As a rule (when the size of the inoculum was just right), the effect of the extract was manifested both by the earlier appearance and greater abundance of the growth. The extracts, with few exceptions, influenced favorably the growth of all the bacteria studied, and the effect varied with the amount of extract added.

Effect of Beef Heart Extract.—The following is a typical experiment with beef heart extract. The extract was prepared from fresh beef heart in the manner outlined, and varying amounts were added to tubes containing 5 cc. of nutrient broth. The results are shown in Table I. Similar results were obtained with the use of agar as a base.

TABLE I.

Effect of Heart Extract on the Growth of Bacteria.

Culture.	Amount of extract.									
	1.0 cc.		0.1 cc.		0.01 cc.		0.001 cc.		0.0 cc.	
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
Meningococcus.....	+*	++	+	++	−	±	−	−	−	−
Pneumococcus.....	++	++	+	++	+	+	−	+	−	+
Streptococcus.....	++	++	−	+	−	−	−	−	−	−
B. diphtheriæ.....	±	+++	−	+++	−	++	−	+	−	+
B. pertussis.....	++	++	++	++	−	+	−	+	−	+
B. influenzæ†	−	+	−	+	−	−	−	−	−	−

* The plus signs indicate the presence and relative abundance of growth.

† Inoculations of the influenza bacillus were made with a 4 mm. loop direct from blood broth cultures; small amounts of blood were thus carried over, but not enough to permit growth in plain broth.

Effect of Extracts of Various Organs and Tissues of the Rabbit and Cat.—A uniform procedure was employed in the following experiments. The animal was etherized, exsanguinated, and the organs or tissues were removed aseptically to sterile Petri dishes. The extracts were made as described. With stomach or intestinal mucosa the organs were first washed free of fecal matter, the mucosa was then scraped off with a scalpel and suspended in nine times its weight of saline solution. Extracts from tissues of four rabbits and one cat were tested in the course of the experiments. The method of testing was the same as that described above.

The results were strikingly uniform. When 1 cc. of the extracts was added, no differences in effect were discernible. Extracts of all the tissues tested had a decidedly stimulating action on the growth of the various test organisms. However, when smaller amounts of the extract were added, certain fairly constant differences were observed. Extracts of liver and stomach mucosa were more effective than those of other tissues, while those of brain and muscle were less so. The influence of other tissue extracts was practically the same. They had somewhat different effects on different bacteria, but that may have been due to the error in technique which is considerable.

Table II gives the results obtained in one series of tests. It is difficult to summarize the data. Attention can only be called to some of the points which cannot be tabulated. The differences between the tubes containing larger and those containing smaller amounts of the extract were always more marked after 24 than after 48 hours. Not only were the colonies on the slants of the former more numerous, but they were, as a rule, larger. The pneumococcus showed a strikingly different behavior on spleen extract agar than on the others. On the former medium autolysis usually occurred 24 to 48 hours after it had appeared in all the other tubes. *Bacillus influenzæ* failed to grow on any of the media until a trace of blood was added. When a drop of a saline suspension of red cells was added to the slant, the growth was practically the same in all the tubes. The results with the gonococci were extremely variable. Even under favorable conditions growth is much slower than that of any of the organisms tested. As a rule, 3 to 4 days elapse before fair sized colonies appear. By that time the tubes are apt to be dry,

and moisture is essential for their growth. They did, however, grow as well on agar containing 0.1 cc. of the extracts as on sheep serum glucose agar.

TABLE II.

Effect of Extracts of Rabbit Tissues on the Growth of Bacteria.

Culture.	Amount of extract.	Extract.								
		Liver.	Spleen.	Kidney.	Heart.	Lung.	Muscle.	Brain.	Testes.	Stomach.
	cc.									
Streptococcus....	0.1	++	++	++	+	+	+	+	+	++
	0.01	±	±	±	±	±	±	±	±	+
	0.0	±	±							
Pneumococcus....	0.1	+++	+++	+++	+++	++	++	++	+++	+++
	0.01	++	++	++	++	++	++	++	++	+++
	0.0	+	+							
B. diphtheriæ.....	0.1	++	++	++	++	++	+	±	±	++
	0.01	+	±	++	±	±	±	Tr.	±	++
	0.0	−	−							
Gonococcus.....	0.1	+	+	++	+	+	+	+	+	++
	0.01	+	+	++	+	+	+	+	+	++
	0.0	−	−							
Meningococcus...	0.1	++	++	++	++	++	+	±	++	+++
	0.01	++	+	+	++	++	+	Tr.	++	+++
	0.0	−	−							

Growth Accessory Substances in Extract of Mucosa of Various Organs.—The interesting fact brought out by the previous experiments was that extract of stomach mucosa stimulated the growth of bacteria more actively than those of other tissues. It was of interest to note whether extracts of other mucosa would have a similar effect. It was also anticipated that these extracts might show some specific selective action. Tests were made with nasopharyngeal, stomach, intestine, and genitourinary mucosa. The usual method of extraction and testing was employed. The results showed clearly that extracts of all the mucosa tested had a favorable effect on growth. No

conclusion could be drawn from these experiments regarding their specificity, because of the large element of error involved in the technique.

Growth Accessory Substances in Cat Tissues.—The object of these tests was to determine whether there was any marked difference in the distribution of the growth accessory substances in different animals. The extracts were made and tested in the usual manner. The only difference in technique was the use of peptone phosphate agar and peptone phosphate broth in place of the beef infusion medium. The former furnished a vitamine-free base, and the effects of the extracts were brought out more strikingly than when infusion media

TABLE III.

Effect of Extracts of Cat Tissues on the Growth of Bacteria.

Culture.	Control.	Extract (0.1 cc. to tube).							
		Liver.	Spleen.	Kidney.	Heart.	Lung.	Brain.	Stomach.	Intestines.
Streptococcus.....	−	++	++	++	++	++	+	++	++
Pneumococcus.....	−	++	++	++	++	+	++	++	++
B. diphtheriæ.....	−	++	++	++	++	++	++	++	++
Meningococcus.....	−	++	++	++	++	++	++	+++	++
B. pertussis.....	−	+	+	±	+	±	±	±	+

were used. Otherwise the results were essentially the same as those obtained with rabbit tissue extracts. As was previously noted, the autolysis of the pneumococcus occurred later on liver and spleen extract media than on the others. The results are shown in Table III.

Effect of Tissue Extracts on Lag.—The phenomenon of lag is of practical as well as theoretical interest. It has been studied extensively by a number of workers, among them Müller, Penfold, and Chesney. The most carefully worked out theory explaining this phenomenon is that advanced by Chesney. He believes that lag represents the time necessary for the bacterial cells to recover from injury.

It seemed of interest to determine the effect of the extracts on the lag phase, or, in other words, the influence of growth-stimulating substances on the speed of cell recuperation. Beef heart and rabbit tissue extracts were used. The streptococcus and pneumococcus served as the test cultures, because of the ease with which they can

TABLE IV.

Effect of Beef Heart Extract on Bacterial Lag.

Test culture, *Streptococcus hæmolyticus*.

Platings.	Time interval.	No. of colonies per cc. of broth.		
		Extract, 1 cc.	Extract, 0.1 cc.	Broth.
	hrs.			
1	0	2,300	3,300	1,300
2	1	3,800	3,900	2,500
3	3	32,000	11,000	1,400
4	5	230,000	14,000	>100
5	6	320,000	43,000	>100
	24	Heavy turbidity.	Sterile.	Sterile.

TABLE V.

Effect of Beef Heart Extract on Bacterial Lag.

Test culture, pneumococcus.

Platings.	Time interval.	No. of colonies per cc. of broth.		
		Extract, 1 cc.	Extract 0.1 cc.	Broth.
	hrs.			
1	0	3,200	3,000	3,200
2	1	3,700	3,200	4,000
3	3	4,000	4,000	4,100
4	5	4,600	4,300	1,300
5	6	15,000	8,000	>1,000
	24	Good turbidity.	Sterile.	Sterile.

be manipulated. To tubes containing 5 cc. of broth were added graded amounts of the extract. Duplicate tubes of broth and extract broth were inoculated with the same amount of a culture suspension. Glucose agar plates were made immediately and at stated

intervals. The plates were incubated at 37°C. for 24 hours, and the number of colonies was counted. The figures in the tables give the average number of colonies per cubic centimeter of broth.

The results are shown in Tables IV, V, and VI. These tables show clearly that beef heart and rabbit tissue extracts have a decided influence on bacterial lag, and, moreover, that the effect varies with the amount of extract added. The smaller the amount of extract, the longer is the lag phase; and when the concentration of the accessory substances is too low, the results are the same as in the broth controls.

Although this is not a study of lag, the bearing of these experiments

TABLE VI.

Effect of Rabbit Tissue Extracts on Bacterial Lag.

Test culture, pneumococcus.

Plating interval.	No. of colonies per cc. of culture at time of plating						
	Stomach extract.		Heart extract.		Muscle extract.		Broth C.*
	0.5 cc.	0.1 cc.	0.5 cc.	0.1 cc.	0.5 cc.	0.1 cc.	
hrs.							
0	15,000	15,000	18,000	17,000	17,000	17,000	18,000
2	22,000	15,000	17,000	17,000	18,000	17,000	18,000
4	38,000	26,000	60,000	25,000	36,000	9,500	14,000
5	71,000	53,000	73,000	46,000	71,000	7,000	5,000
6	180,000	90,000	148,000	87,000	210,000	4,500	7,000
24	+++	+++	+++	+++	+++	Sterile.	Sterile.

* Two broth controls were run with the same results.

on the explanation of this phenomenon should be noted. The fact that even under favorable conditions there is a quiescent period before active growth commences, confirms Chesney's assumption of recuperative adaptation. It appears, however, that recuperation is not possible unless some substances are present which favor the active initiation of growth. Ordinarily this is apparently supplied by the disintegrating cells. This interpretation would account for the need of heavy inocula for successful transplants of cultures.

Besides throwing some light on the lag phenomenon, these experiments confirm the fact that the tissue extracts contain some

substances favorable to bacterial growth. All of them in certain concentrations convert an unfavorable substrate where growth does not occur into a favorable one. This effect is produced in the case of rabbit heart and stomach extracts by the addition of only 0.1 cc., while in the case of muscle extract of the same animal a larger amount must be added to obtain that result. The influence of the extract must, therefore, depend on the concentration of some substance or substances in them. Whatever the nature of these substances, it is of interest to note that they are present in lesser concentration in extract of muscle than in that of heart or stomach mucosa.

Effect of Nasal Secretions on Growth of Bacteria.—Shearer has reported experiments showing the stimulating action of nasal secretions on the growth of the meningococcus. It seemed worth while, in view of the results recorded above, to confirm and possibly extend these observations. The tests were limited to nasal washings and saliva.

The first tests were made with nasal secretions. These were mainly confirmatory of the work of Shearer. The secretions were obtained by blowing the nose into strips of sterile gauze. These were put into saline solution or alcohol, and kept for 3 days. The saline extract was divided into two parts, one of which was filtered through a Berkefeld candle, while the other was autoclaved. The alcoholic extract was desiccated to dryness, and then taken up in saline solution.

These extracts were added in 1 cc. and 0.5 cc. amounts to tubes containing 10 cc. of glucose agar and 1 cc. of sheep serum, and plates were poured. The plates were sown with broth suspensions of meningococci; blood and serum plates served as controls. Both saline extracts were superior to the alcoholic extract, though the latter improved the growth as compared with the serum plate. The filtered extract was more effective than the heated one. In no case was the growth so good as on the blood agar plate. The results showed clearly that the nasal mucus contains some substance which favors growth.

The subsequent experiments were modified with a view to determining whether pathogenic bacteria were capable of growing in the

secretions, and, if so, whether there was any difference in their ability to do so. For this purpose saline washings of the nose of apparently normal individuals as well as saliva were used; 50 cc. of warm saline solution were used for each washing. These were collected in sterile bottles, filtered through a Berkefeld candle, tested for sterility, and tubed in 2 cc. amounts in small test-tubes. The saliva was collected in the course of the day, filtered through a Berkefeld candle, and tubed in the same way.

Growth in Nasal Washings.—The nasal washings of five adult individuals and one rabbit were used. The washings were all ob-

TABLE VII.

Growth of Pathogenic Bacteria in Nasal Washings.

Source of washings.	Culture.															
	Meningococcus.				Gonococcus.		Strepto-coccus.		Pneumo-coccus.		*B. diph-theriæ.*		*B. per-tussis.*		*B. influ-enzæ.*	
	Para.		Normal.													
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.	24 hrs.	72 hrs.
Rabbit.	−	−	−	−	−	−	+	+	+	+	−	−	+	+	−	−
W.	+	+	+	+	−	±	+	+	+	+			+	+	+	+
D.	−	−	−	−	−	Tr.	+	+	+	+	−	−				
Z.	+	+	−	−	−	Contaminated.			+	+	±	±			+	+
K.	+	+	+	+	−	"	+	+	+	+	−	−	+	+	+	+
T.	−	−	−	−	−	±	+	+	+	+	+	+	+	+	−	−
Saliva K.	−	−	−	−	−	−	−	−	−	−	±	+	−	−	−	−
" Z.	−	−	−	−	−	−	−	−	−	−	±	+	−	−	−	−

tained on the same day and treated in the same manner. The inoculations were made by diluting 0.1 cc. of a 6 hour broth culture in 5 cc. of broth and adding 0.1 cc. of the suspension to each tube. Growth was determined by turbidity and checked by means of Gram-stained films. The results are shown in Table VII.

The washings were made as nearly as possible in the same manner. The rest of the procedure was identical. The same cultures were used for inoculating all the tubes. Yet there were marked individual and specific differences. A given organism grew in the washings of one individual and not in those of another. Chemical tests revealed no differences in the washings. They were all slightly acid

when fresh, but reverted to alkalinity on standing (probably due to a loss of carbon dioxide). No amino nitrogen and only a trace of nitrogen could be detected in 5 cc. of the washings. Reduction tests for glucose were also negative. Nevertheless they offered a favorable substrate for growth while saline controls were negative. No growth appeared in the saliva tubes. Evidently the nasal secretions of some individuals contain substances which actively stimulate growth, while saliva does not.

Relative Effect of Saline and Ether Extracts.—To ascertain the relative importance of the water-soluble and fat-soluble fractions, com-

TABLE VIII.

Growth Accessories in Ether and Saline Extracts.

Extract.	Culture.					
	Streptococcus.	Pneumococcus.	*B. diphtheriæ.*	Meningococcus. Normal.	*B. pertussis.*	*B. influenzæ.*
Saline	+	+	++	+	±	−
Ether	±	−	±	±	−	−
Mixture	+	+	+	+	±	−
Peptone	−	−	±	−	−	−

parative tests were made with saline and ether extracts. Blood clot was used for this experiment; it is rich in vitamines and has a markedly stimulating effect on the growth of bacteria. Equal portions of blood clot, from which the serum had been completely removed by centrifugalization, were weighed out. One portion was suspended in ten volumes of saline solution, the other in ten volumes of alcohol ether (1:3). The alcohol was added first to desiccate the clot and the ether was then added. The saline extract was filtered through a Berkefeld candle. The ether extract was decanted, evaporated nearly to dryness under suction, and the residue taken up in an amount of saline solution equal to the original volume.

Media containing these extracts were then prepared. This time a peptone phosphate solution was used as a base in place of nutrient broth to eliminate all traces of vitamines from other sources. 1 cc. of the respective extracts, as well as a mixture of the two, was added to tubes containing 5 cc. of the peptone solution. These were inoculated in the usual manner and incubated at 37°C.

The results are tabulated in Table VIII. It is evident that the saline extract contains some substances which favor the growth of bacteria, while the ether extract is practically devoid of them. Moreover, inoculations of the various organisms made into the extracts directly failed to give growth. Neither the extract alone nor the peptone alone was capable of supporting growth. The two together constituted a favorable medium. Similar results were obtained with saline and ether extracts of beef heart.

Nature of the Growth Accessory Substances in These Extracts.—The question naturally arises: To what is the favorable effect of these extracts due? Is the improvement of the medium attributable to the addition of food accessory substances or merely to an enrichment in its nutritive quality? This question is naturally difficult to answer, but some experiments have been made which indicate that we are dealing with a vitamine stimulation.

1. If the effect of the extracts is the result of a high content of nutritive substances, bacteria should grow abundantly in them. Tests showed that although moderate growth occurred in the concentrated extracts, none appeared when the extract was diluted five times with saline solution.

2. It has been assumed that the addition of serum enhances the value of the medium, because of the "native" protein. The proteins of the extract were precipitated with alcohol and removed by centrifugalization. The supernatant fluid freed from the alcohol by evaporation under vacuum had the same effect as the original extract.

3. There is some contradiction in the literature regarding the heat stability of vitamines, but it is generally agreed that high temperatures partially or completely destroy these substances (Chick and Hume). On the other hand, amino-acids or carbohydrates, the essential nutritive substances for bacteria, are not affected by heat.

Extracts were prepared in different ways, by employing heat, and the effect on growth was compared with those prepared at the ice box temperature. Lean fresh beef heart was used. Equal weights were macerated and taken up with nine volumes of saline solution. These suspensions were treated respectively as follows: (1) kept in ice box over night and steamed in an Arnold sterilizer for 1 hour;

(2) kept in ice box over night and filtered through a Berkefeld candle; (3) kept at 55°C. over night and heated in an Arnold sterilizer for 1 hour; (4) kept at 55°C. over night and filtered through a Berkefeld candle; (5) extracted by boiling for 1 hour and sterilized in an Arnold sterilizer for 1 hour; (6) extracted by boiling for 1 hour.

The relative effect of these six extracts was tested in the usual way. The results with the agar slants were more striking than those obtained with the broth and are shown in Table IX.

TABLE IX.

Effect of Heat on the Concentration of Substances Favoring the Growth of Bacteria.

Agar slants. Relative abundance of growth after 24 hrs. at 37°C.

Extract.	Culture.					
	Strepto-coccus.	Pneumo-coccus.	*B. diphtheriæ.*	Meningococcus.		*B. pertussis.*
				Normal.	Para.	
Ice-Arnold	++	++	−	±	−	±
Ice-filter	++++	+++	++	+++	++	+
55°C.-Arnold	+	+	−	±	−	±
55°C.-filter	++++	++	++	+++	++	+
Boiling-Arnold	+	+	−	±	−	±
Boiling	++	+	±	±	−	±
Plain agar	+	+	1 colony.	±	−	−

The results shown in Table IX indicate clearly that heat destroys to some extent the substances which favor bacterial growth. Extracts prepared at ice box temperature or at 55°C. are superior to those obtained by heating, and the longer the heating, the less effective are the extracts. The fact that these substances are heat-labile supports the view that they belong to the class of growth accessory substances.

SUMMARY.

The growth of all the pathogenic bacteria studied was favorably influenced by the addition of small amounts of tissue extracts.

Beef heart, rabbit and cat tissues, and human nasal secretions contain substances favorable to the growth of the organisms tested. The mucosa of different organs, spleen, liver, and kidney, are relatively rich in these substances, while muscle is relatively poor. The favor-

able effect of the extracts is manifested by an enhancement of growth and a reduction of lag.

The water-soluble substances are apparently the ones essential for bacterial development; the ether extract has no effect on growth.

Experiments are reported which indicate that the substances in question belong to the class of so called vitamines.

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STUDIES ON ORGAN TRANSPLANTATION.

I. Transplantation of the Thyroid Gland with Intact Blood Supply.

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Plates 4 to 6.

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There are two methods of free grafting of a gland: the transplantation of pieces of tissue, and the transplantation of the whole organ with anastomosis of its blood vessels to those of the same person or of another person. In 1892 von Eiselsberg transplanted the thyroid gland in a cat. He removed half of the gland and transplanted it into the abdominal wall. Several weeks later he extirpated the other half of the gland. The animal remained in good condition but died when the transplanted thyroid was removed. Since that time many instances of thyroid grafting, both experimental and clinical, have been reported, and it has been shown that thyroid grafts in animals take and functionate.

Fairly effective results were obtained by Cristiani (1904, 1906) after transplantation of thyroid tissue in the subcutaneous cellular tissue, by Payr (1906) after transplantation in the spleen, and by Kocher after transplantation in the bone marrow. On the other hand, there are also many reports of negative results. Enderlen considered the functional activity of transplanted thyroid limited and temporary, as a consequence of his experiments in which cats and dogs died even after a period of 6 months. Bircher, Leichner and Köhler, Stieda, and others implanted thyroid gland into the subcutaneous tissue or bone marrow of patients, with no permanent improvement. In 1914 von Eiselsberg reported the transplantation of thyroid and parathyroid in man. It seemed to him at first that the grafting was effective, but all efforts finally proved unavailing. He believes that the function of the transplanted pieces of glands is probably only temporary.

The development of vascular surgery has made it possible to transplant whole organs with their related vessels. In 1905 Carrel and Guthrie extirpated the thyroid gland of a dog, placed it in isotonic salt solution for a few minutes,

and replaced it into the neck, anastomosing the blood vessels but with reversal of the circulation. The arterial blood entered the gland through the thyroid vein and the venous blood flowed from the gland to the jugular vein through the thyroid artery. 11 days after the operation an exploratory opening of the neck was made. The color and consistency of the gland were normal. 8 months after the operation the gland was normal in size and consistency. Later, Carrel (1909) extirpated a large parenchymatous goiter in a dog and replanted it. He then cut transversely the common carotid and united its central end to the peripheral end of the internal jugular. The central end of the jugular was sutured to the peripheral end of the carotid. The size of the gland increased enormously. At the same time, clear fluid exuded from the surface of the gland but diminished and stopped completely after more than a week.

Stich and Makkas performed a series of thyroid transplantations. After the dissection of the upper thyroid artery to the point of its departure from the common carotid, they resected a rhomboid flap of the carotid in connection with the thyroid artery and inserted it in the corresponding hole of the other carotid. The internal jugular vein was cut transversely just beneath its union with the lower thyroid vein, and sutured with the external jugular vein by end to side or end to end anastomosis. At the same time the other half of the gland was extirpated to obtain, immediately, sufficient function for the transplanted thyroid. Of three autoplastic transplantations, two were positive; that is, 51 and 245 days respectively after the operation the circulation was intact and the gland, both macroscopically and microscopically, was normal.

The gland was transplanted seven times homoplastically, but no positive result was obtained. An obstruction was almost always found at the point of the venous anastomosis, which brought about necrosis and reabsorption of the gland.

Borst and Enderlen similarly transplanted the thyroid of dogs and goats. Their method differed somewhat from the procedure employed by Stich and Makkas in that they used, for the grafting of the thyroid artery, the complete segment of the carotid with the thyroid artery. In seven instances they extirpated the thyroid and replanted it in the same animal. In two of the seven animals (20 and 122 days after operation) the glands were in good condition. In the other animals necrosis, hemorrhagic infarct, degenerative metamorphosis, etc., took place, due to the thrombosis of the thyroid vein. They attempted homoplastic transplantation seven times (in dogs and goats), but none was successful. They also attempted in three instances to transplant the thyroid from man to man by the employment of blood vessel sutures. The upper pole of the gland was transplanted into the axilla or the elbow. The results in every case were fruitless.

Goodman also investigated this problem. He made a biterminal suture of a segment of the attached carotid of the severed vessel of the host, and an end to end suture of the thyroid vein with the central end of the external jugular of the opposite side. He performed three autoplastic and twenty-seven homoplastic transplantations, and observed them from 24 hours to 112 days after the operation. In autoplastic transplantation he succeeded in two instances (the dura-

tion of life was 4 and 23 days respectively) in retaining the thyroid gland in its normal state; while in homoplastic transplantation the gland remained intact for a short time only, and then showed evidence of absorption.

The results of the investigations with regard to thyroid transplantation as reviewed in the literature did not seem uniform and we were therefore induced to investigate the problemf urther. We chose for our experiments the transplantation of the gland through blood vessel sutures because of the favorable restoration of the circulation of the gland by this method.

EXPERIMENTAL.

All the experiments were performed on dogs. Since the relation of the thyroid to its vessels in the dog is different from that in man, before describing the technique it may be well briefly to note this difference. In a dog the glands are spindle-shaped, situated beside and beneath the larynx, usually separated from each other, and more or less covered with muscle. The dog, especially the bulldog, has a relatively large thyroid, which is not always proportional to the size of the animal; a small dog may have remarkably large glands due to the development of the so called colloid goiter. The arterial blood reaches the gland mainly through the superior thyroid artery, which arises from the common carotid and enters the gland at its upper pole after having made an upward convex curve. Very rarely this artery enters the gland near its middle and ramifies with many branches. The inferior thyroid artery is very small; accordingly it may usually be ignored in the transplantation of the thyroid. The venous blood flows out through two veins, the superior and inferior thyroid veins. The superior thyroid vein leaves the upper pole of the gland and empties into the internal jugular vein, while the inferior thyroid vein, consisting of two stems, opens into the same vein further down. The diameter of the superior thyroid artery and thyroid veins is scarcely greater than 1.5 to 2.5 mm. It is, therefore, almost impossible to anastomose these vessels.

The operation was performed under intratracheal ether anesthesia. The Carrel (1907) technique of blood vessel suturing was employed, and special attention was paid to rigid asepsis. A longitudinal median

incision was made in the neck and the superficial muscles were separated. The thyroid gland was dissected from the surrounding tissue, the superior thyroid artery and superior and inferior thyroid veins being left intact. In most instances the gland was extirpated with a portion of the internal jugular vein and a segment of the common carotid artery, connecting the thyroid veins and the superior thyroid artery respectively. The gland was immediately wrapped in a sponge saturated with warm salt solution. After a few minutes the gland was transplanted to the other side of the neck of the same dog, or into the neck of another dog, where the thyroid had been previously removed. The segment of common carotid was inserted in the place of the other below the point of outlet of the thyroid artery. The peripheral end of the internal jugular vein was united to the central end of the internal or external jugular vein of the recipient by end to end anastomosis. In one case the gland with a rhomboid flap of the carotid at the point of outlet of the superior thyroid artery was removed (Stich's so called patching method) and replaced in the wound in the neck. In two animals the peripheral end of the internal jugular vein was united to the wall of the external jugular vein (end to side). Moreover, in two cases, after resection of half of the spleen, the thyroid was transplanted to the splenic vessels of the same animal. In these instances the superior thyroid artery was sutured to the splenic artery, and the internal jugular vein to the splenic vein by end to end anastomosis. The time required for the extirpation of the gland and its complete transplantation was usually from 1 to 2 hours. The clamp on the vein was removed first and then that on the artery. As soon as the clamps were unfastened the gland became normal in color; it was somewhat distended. Several days after the operation the wounds were opened and the condition of the transplanted thyroid gland was examined. As a rule, if the gland appeared normal the other intact thyroid was fixed in 10 per cent formaldehyde for microscopic examination.

Both autotransplants and homotransplants of the gland were made, as shown in Table I. A total of eight autoplastic and seven homoplastic transplantations was made. Two autoplastic transplants (Experiments 2 and 3) and one homoplastic (Experiment 9) are described below.

Experiment 2 (*Dog 2*).—Adult bulldog, male; weight 12 kilos.

Sept. 18, 1918. Placed under ether anesthesia and the right thyroid gland transplanted to the left side of the neck. The segment of the right carotid artery, from which the superior thyroid artery arose, was implanted by end to end anastomosis into the left carotid. The peripheral end of the right internal jugular vein was anastomosed to the cardial end of the left external jugular vein. The excised gland had been left in a salt pack for a short time. After the removal of the clamps the pulsation of the superior thyroid artery was very evident and the circulation through the gland was reestablished so that it immediately became normal in color. The extirpation of the gland and its complete transplantation occupied 1½ hours. The wound was closed according to the routine technique of the laboratory.

Sept. 20. The animal was in good condition and had a normal appetite.

Oct. 9. Second operation. The weight of the animal at this time was 13.1 kilos. The transplanted thyroid appeared perfectly normal. The intact left thyroid was removed. The animal recovered from the operation and never showed any signs of tetany.

Oct. 29. The animal died early in the morning (41 days after the first operation and 20 days after the second).

Autopsy.—Performed shortly after death. The wound had healed completely; the cause of death could not be found. The transplanted thyroid was normal in size, color, and consistency and appeared normal on section. The capsule of the gland was not markedly thickened. The implanted blood vessels were patent. The lines of union were covered with epithelium and so smooth that they were found with difficulty.

Microscopic Examination.—The transplanted thyroid showed no difference from the control gland. The capsule of the gland was not increased in thickness. The follicles were normal in size and about the same in both the central and peripheral parts of the gland; they were lined with cuboidal epithelium, and filled with a normal amount of colloid. The staining reaction was normal; mitoses were not present. The interstitial connective tissues were not increased as compared with the control specimen. Increase of blood vessels was not visible. No leucocytic infiltration could be found (Figs. 1 and 2).

Experiment 3 (*Dog 3*).—Adult collie, female; weight 21.3 kilos.

Sept. 19, 1918. Etherized and the left thyroid gland transplanted to the right side of the neck. The gland was small, as was also the internal jugular vein. The left thyroid was displaced to the right with a segment of the common carotid and the internal jugular vein. The segment of the left carotid was inserted into the right carotid. The peripheral end of the left internal jugular vein was implanted into the wall of the right external jugular by a terminolateral anastomosis. The operation was difficult owing to the different sized vessels. Nevertheless, it was, on the whole, satisfactory. There was no leakage at the line of suture. The circulation was reestablished about 1½ hours after the extirpation of the gland. The wound was closed in the routine manner.

TABLE I.

Summary of Experiments.

Experiment No.	Operation.	Duration of observation.	Results.			Remarks.
			Condition of blood vessels.	Condition of transplanted thyroid.	Microscopic examination.	
			Autoplastic transplantation.			
	1918	*days*				
1 (Dog 1).	Sept. 11. Transplantation of right thyroid with a segment of common carotid to left side. Oct. 30. Exploratory opening of neck.	49	Left common carotid pulsating everywhere; superior thyroid artery and inferior thyroid vein thrombosed.	Entirely absorbed.		At first operation great hemorrhage from peripheral end of right common carotid due to slipping ligature. Lower part of wound slightly infected. Dog still living.
2 (" 2).	Sept. 18. Transplantation of right thyroid with a segment of common carotid to left side. Oct. 9. Extirpation of the original left thyroid.	61	Arterial and venous lumen patent. Line of union covered with endothelium, and smooth. No thrombosis or clots.	Size, color, and consistency absolutely normal.	No necrosis. Appeared normal.	Oct. 29. Died.
3 (" 3).	Sept. 19. Transplantation of left thyroid with a segment of common carotid to right side. Terminolateral anastomosis between left internal and right external jugular vein.	62	Arterial and venous lumen patent. Line of union smooth. No thrombosis or clots.	Appeared entirely normal.	No necrosis. Appeared almost normal.	Nov. 30. Died.

4 (Dog 4).	Sept. 21. Transplantation of left thyroid with a segment of common carotid to right side. Terminolateral anastomosis between left internal and right external jugular vein.	4	Arterial anastomosis in good condition. Very small clot at mouth of external jugular vein.	Almost same size as the other. Fairly dark and hard but mostly translucent.	Almost normal with exception of a few congested blood vessels.	Both thyroids small. Congestion of venous blood at the point of anastomosis due to a bend of transplanted internal jugular vein which was too long. Animal died of distemper.
5 (" 5).	Sept. 23. Replacement of left thyroid. Rhomboid flap was taken from left common carotid. Oct. 9. Exploratory opening of the neck.	18	Arterial suture intact, but vein stopped up.	Normal size; soft. Surface of section pale, not translucent.	Follicle and colloid entirely disappeared. Abundant leucocytic infiltration and proliferation of connective tissue.	Both thyroids very small. Oct. 11. Died.
6 (" 6).	Nov. 29. Replacement of right thyroid with a segment of right common carotid	1	All sutures in good condition.	Appears normal.	Almost normal; a few leucocytic infiltrations.	Nov. 30. Died. Cause of death unknown.
7 (" 7).	Dec. 16. Resection of half of spleen. Transplantation of left thyroid into splenic vessels. Jan. 13, 1919. Exploratory laparotomy.	28	Obstruction at point of arterial anastomosis.	Normal size but dark in color. Whole gland necrotic.	Necrosis.	Superior thyroid artery entered at the middle part of the gland.

TABLE I—*Concluded.*

Experiment No.	Operation.	Duration of observation.	Results.			Remarks.
			Condition of blood vessels.	Condition of transplanted thyroid.	Microscopic examination.	
	1918	*days*				
8 (Dog 8).	Dec. 23. Transplantation of right thyroid into splenic vessels. Jan. 13, 1919. Exploratory laparotomy.	21	Obstruction at point of arterial anastomosis; thrombosis in vein.	Gland diminished to size of tip of thumb. Surface of section anemic; no blood came out.	Necrosis.	At the first operation spleen not resected.
	Homoplastic transplantation.					
9 (Dog 9).	Sept. 25. Transplantation of left thyroid with a segment of common carotid of left neck. Internal jugular vein united to external jugular.	6	Line of suture in good condition. Caliber of artery and vein patent.	Size, color, and consistency almost normal. Longitudinal section showed a thin brownish and somewhat gelatinous part along the posterior border.	Almost normal. Septum between follicles increased more or less in thickness. Follicles filled with colloid. Deposit of lime and loosed epithelium visible here and there in follicles.	Both glands very large. Oct. 1. Animal died of distemper.
10 (" 10).	Sept. 26. Transplantation of left thyroid with a segment of common carotid to right neck. Ends of internal jugular vein united to each other by end to end anastomosis. Oct. 14. Exploratory opening and proof excision of the gland.	18	Right common carotid pulsating everywhere.	Gland kept its original size; color pale red. No hemorrhage on incision.	Interstitial tissues increased. Follicles filled with leucocytes, detritus, and fibrin. Here and there deposit of lime.	

11 (Dog 11).	Sept. 27. Transplantation of right thyroid with a segment of common carotid to left neck. Oct. 14. Exploratory opening. Extirpation of right intact thyroid.	24	Thrombosis in common carotid. Obstruction of internal jugular vein along the suture line.	Gland kept almost original size; softer than normal. Cut surface brown, dim, not translucent.	Capsular thickening. No trace of follicles or colloids.	At second operation the veins on anterior portion of gland were visible and filled with blood. Oct. 21. Animal died with typical symptoms of tetany.
12 (" 12).	Sept. 28. Transplantation of left thyroid with a segment of common carotid to left neck.	63	Transplanted common carotid and internal jugular vein obstructed.	Entirely disappeared.		A week after first operation large hematoma formed in the neck.
13 (" 13).	Sept. 30. Transplantation of left thyroid with a segment of common carotid to left neck.	5	Thrombosis in common carotid and inferior thyroid vein.	Enlarged; cut surface dark red.	Septum increased in thickness. Follicles filled with detritus and fibrin. Nuclear staining weak.	Oct. 5. Died.
14 (" 14).	Oct. 1. Transplantation of right thyroid with a segment of common carotid to left neck. Oct. 30. Exploratory opening.	29	Thrombosis in common carotid.	Atrophy of gland to size of tip of little finger.		Caliber of internal jugular vein very small.
15 (" 15).	Oct. 4. Transplantation of right thyroid with a segment of common carotid to left neck Oct. 30. Extirpation of right intact thyroid.	27	Thrombosis in common carotid, in inferior thyroid vein, and in internal jugular vein.	Diminished to size of tip of thumb. Consistency soft.	Follicles and colloid entirely disappeared. Here and there clumps of lymphocytes. Abundant proliferation of connective tissue.	Animal died with symptoms of tetany day after second operation.

Oct. 9. Second operation. At this time the animal weighed 21.4 kilos. The transplanted thyroid gland appeared normal. The intact right thyroid was removed and fixed in formaldehyde.

Nov. 20. The animal died during the preceding night (62 days after the first operation and 42 days after the second).

Autopsy.—Performed shortly after death. The wound had healed by first intention; there were slight adhesions at the site of operation. The transplanted thyroid appeared entirely normal. The blood vessels were patent, and the site of anastomosis was in excellent condition. The cause of death could not be determined.

Microscopic Examination.—The transplanted thyroid was practically normal. The thickness of the capsule of the gland was normal. No difference was seen in the size and form of the follicles, in the colloid content, or in the quantity of interstitial connective tissue, in comparison with the other gland of the same dog. Cuboidal epithelium lined the follicles as in the control section. The gland contained a large number of blood vessels. Here and there clumps of a few leucocytes were visible (Figs. 3 and 4).

Experiment 9 (Dog 9).—Adult mongrel, male; weight 5 kilos.

Sept. 25, 1918. The left thyroid of another dog was transplanted, under ether anesthesia, to the left side of the neck by the insertion of a segment of the common carotid into the carotid. After the dissection and removal of the left thyroid gland, about 3 cm. of the left common carotid were resected. The peripheral end of the internal jugular vein of the donor was united to the central end of the external jugular of the recipient. The transplanted thyroid was very large, as was also the internal jugular vein. The operation was easily performed. After 1¼ hours the circulation through the gland was reestablished satisfactorily. Closure of the wound was made as usual. The animal recovered from the operation but developed distemper.

Oct. 1. The animal died during the night.

Autopsy.—Death due to distemper. Both the arterial and the venous anastomoses were in good condition; the caliber of the vessels was well preserved. The transplanted thyroid appeared normal in size, color, and consistency. On section it was normal in appearance except in the posterior border where the tissue was somewhat brown in color and was more gelatinous than the other part.

Microscopic Examination.—The capsule of the gland was more or less thickened. The interlobular septum also appeared to be thickened. The size of follicles and their colloid content were as well preserved as in the autografts. The staining reaction appeared normal. Few follicles contained desquamated cells in their lumen. Some of these cells were calcified. There was a small amount of leucocytic infiltration. The vascularization of the gland was normal (Fig. 5).

Eight autoplastic and seven homoplastic transplantations of the thyroid gland were performed. In two of the eight autoplastic transplantations the gland was transplanted to the splenic vessels, in six

to the neck. The first two cases were examined by exploratory laparotomy 21 and 28 days respectively after the first operation. It was found that the glands had become necrotic because of thrombosis. It seems almost impossible to get successful results by anastomosing such a small vessel as the superior thyroid artery to the splenic artery. Two of the six transplantations to the neck were replaced in the same wounds, and four were made to the other side of the neck. One of these was examined 18 days after the operation and obstruction of the transplanted vein and necrosis of the gland were found. One transplant was examined 49 days after the operation. The gland had entirely disappeared, and the superior thyroid artery and the inferior thyroid vein could not be found, though the pulsation of the common carotid was very evident throughout. The cause of the failure was due to infection of the wound. In four instances (1, 4, 41, and 62 days after the operation), all blood vessels were patent, and the gland appeared, both macroscopically and microscopically, to have preserved its normal structure.

Seven of the homoplastic transplantations were examined in periods of 5, 6, 18, 24, 27, 29, and 63 days after the first operation. In two the segments of the carotid remained free from thrombosis and were covered smoothly with endothelium, although in five instances the transplanted vessels, in whole or in part, were occluded. The thyroid gland showed evidences of remarkable change in proportion to the time elapsed. 6 days after the operation the gland retained its original size and, for the most part, was translucent, but in a part of the posterior border the tissue was beginning to soften. Microscopic examination showed that the size of follicles and colloid content were as well preserved as in the autograft. In the gland which was examined 18 days after the transplantation, the consistency was markedly soft, the color of the section was pale red, and no hemorrhage had occurred at that time, notwithstanding the fact that the gland kept its original size and the carotid was still patent. Microscopic examination showed great degeneration of the gland. After 24 days the microscopic change of the gland increased, and it became gradually smaller. In one case, 63 days after the operation, no trace of the gland was to be found. In brief, our attempts at homoplastic transplantation were unsuccessful.

DISCUSSION.

Our results in thyroid transplantation by blood vessel sutures agree on the whole with the experiences of Stich and Makkas, Borst and Enderlen, and Goodman. In the autoplastic transplantations we succeeded, as they did, in maintaining the glands for a long time in their original shape and structure, and apparently in function. In the series of homoplastic transplantations, on the contrary, the transplant remained intact for a time, then underwent degenerative changes, and, consequently, absorption.

Hesselberg has published a paper concerning a comparison of autoplastic and homoplastic transplantation of thyroid tissue in guinea pigs. She transplanted pieces of glands into the subcutaneous tissue of the abdominal wall and examined them at intervals of from 24 hours to 52 days. In the first stage, comprising the first 4 to 5 days, there was no noticeable difference between the autografts and the homografts. Large parts of both grafts became necrotic in the center, but in the peripheral part only a narrow zone of thyroid tissue was left. In the second stage, extending over the next 7 days, the differences between the two grafts appeared. There was an increase in the number of follicles in the autografts and a corresponding decrease in the size of the necrotic central area. In the homografts a large number of lymphocytes and connective tissue were visible. From the 12th day on, in the third stage, the difference between the grafts was sharply defined. In the autograft the regeneration of the thyroid tissue was steadily progressing and nearly completed after 21 days. In the homograft, on the contrary, the destruction of the follicles progressed with great intensity.

Loeb (1918–19, *a*, *b*) recently designated the transplantation into nearly related animals and to the nearly related of the same species as "syngenesioplastic" transplantation. He carried out transplantation of thyroid from mother to children, from sister and brother to sister or brother, and in one instance from child to mother in guinea pigs. The results were intermediate between those which Hesselberg obtained after autotransplantation and homotransplantation. In most instances the thyroid behaved, for a time, like an autotransplanted tissue, but gradually an intensive lymphocytic infiltration took place with secondary destruction of the healthy acini. In a smaller number of cases the fibrous tissue also was increased, the fibroblasts behaving like those in cases of homoplastic transplantation.

In one of our cases of homoplastic transplantation also (Experiment 9) the gland showed indications of degeneration on the 6th day after the operation, in spite of the fact that the transplanted blood

vessels were patent. The destruction of the gland increased in the process of time.

Homoplastic transplantation, however, can be successfully made in certain kinds of tissue. At the instant the animal dies the tissues, in general, do not lose their vitality in spite of the arrest of the circulation. On the other hand, individual tissues have a different power in regard to regeneration and repair. Some tissues, such as skin, fascia, periosteum, and bone, which survive for a long time under the interruption of the circulation, can regenerate and repair easily. Such tissues, as is well known, can be transplanted homoplastically with success although the ultimate fate of the transplant is not fully known.

As has been noted, the opinions of authors differ with regard to the fate of the more highly organized tissues, such as the thyroid gland. Cristiani has shown that transplanted and functionating thyroid may be kept in good condition in its new location for a long time, provided the operation is performed with certain precautions. For the successful transplantation, he emphasized that the pieces of gland transplanted must be small and numerous, that thyroid tissue from the same species only will grow, and that living tissue may be transplanted, provided that not more than 10 seconds elapse between the extirpation of the tissue and its transplantation. Cristiani applied also, clinically, the results of his experimental work, and from his experience on patients arrived at the following conclusions. With his method of thyroid grafting it is possible to produce new thyroid organs which not only retain their vitality and functionate, but gradually grow so that small grafts in time become new thyroid glands (*glandulæ neothyreoideæ*) of different sizes.

Payr (1906) transplanted thyroid gland into the spleen of animals, believing that spleen is suitable soil for healing because of its vascular nature. Of 48 experiments of autoplastic transplantation, mostly on cats and dogs, he obtained eight promising results. The longest duration of the observation was 271 days. He had likewise made the transplantation of the thyroid gland clinically. A few years ago he reported seven cases of thyroid transplantation for myxedematous patients; they showed marked improvement, but most of them relapsed from 3 to 8 years after the operation. He remarked that organic cerebral defects of patients cannot be improved by thyroid grafting.

Kocher reports several favorable cases of transplantation of pieces of thyroid in patients, using the bone marrow as new soil. He prefers the tibial metaphysis to the spleen on account of the greater ease and safety of the operation and the equally vascular soil; hemostasis is secured by operating in two stages. With regard to the tissue for transplantation pieces from exophthalmic goiter are especially desirable, because the glands are remarkably vascular with comparatively firm structure, and because their proper parenchyma surpasses considerably the supporting substance. He stated that there are no grounds for the belief, based on animal experiment, that homoplastic transplantation cannot also succeed in man.

There are some reports of conflicting opinions with reference to these favorable results. Bircher implanted pieces of thyroid into the subcutaneous tissue of the occipital region in three cretins. After short improvement there was relapse into the former state. On histologic examination of the grafts he found that no normal thyroid tissue was left, but necrosis and connective tissue had replaced the whole. His observations made him skeptical as to the permanent function of homoplastic implanted thyroid. Leichner and Köhler were of the same opinion as Bircher. Stieda reported three cases in which the grafting was done on myxedematous children who were more or less idiotic. After operation they showed signs of remarkable, rapid improvement, which shortly came to a standstill. Schaack and Müller repeatedly transplanted thyroid tissue into the bone marrow or into the subcutaneous tissue, with no permanent improvement. The thyroid grafts which Worobjew and Perimow made on a myxedematous patient healed, but subsequently became smaller and were totally absorbed. Von Eiselsberg (1914) also, from his experience with patients, expressed doubt that successful results could be obtained.

Thus, while some observers admit the feasibility of thyroid grafting, others deny it. The negative results of our experiments do not warrant denial of the positive results of Cristiani, Payr, Kocher, etc., but, as Bircher has already noted, we should not lightly pass over the possibility that implanted pieces of tissue will have an influence as long as the colloid exists, that its absorption occurs very slowly, and finally, that the moment the colloid is absorbed its influence stops. Furthermore, although many successful cases of piecemeal homoplastic transplantation on patients have been reported, they are not always without objection, because one important factor is wanting in all; namely, the subsequent removal and microscopic examination of the tissue. In short, for permanent good results we must expect to work further in this direction. Several points must be considered in the successful take of transplanted tissue: (1) The avoidance of

injuring the tissue, and a sufficient blood supply. In this connection the transplantation with intact blood supply is the better method. (2) In the organ transplantation, especially by means of blood vessel sutures, aseptic precaution must be observed as rigidly as possible. If not, the occlusion of the transplanted vessels or necrosis of the organ may easily occur. Concerning asepsis in operations on blood vessels Carrel (1907) states: "It seems that the degree of asepsis under which general surgical operations can successfully be performed may be insufficient for good results in vascular operations." In our first case of autoplastic transplantation the results immediately after the operation were satisfactory, but the lower part of the wound was, unfortunately, slightly infected. At the exploratory opening of the neck, 49 days after the operation, we found that the transplanted common carotid was patent, but that the gland had entirely disappeared, due to infection.

Besides, the rapid work, keeping the gland warm, the avoidance of dryness, rough handling, chemical irritation, close contact with the new soil, and strict arrest of hemorrhage, are all apparently necessary conditions for a good transplantation. If these precautions are used the autoplastic transplantation is almost always successful. But we failed, as did the others, in the homoplastic transplantation, notwithstanding the fact that we used similar care. The factors that may have been responsible for the failure are briefly the following.

It is well known that nerves have some control over the nutrition and function of tissues. Raynaud's disease, for example, is supposed to be due to spasm of the arterioles, resulting from peripheral neuritis. If a nerve trunk is injured severely and the peripheral end undergoes degeneration, several phenomena may occur, due to the loss of the trophic function of the nerve, such as dryness of the skin, trophic ulcers, fragility of the nails and hair, arthropathies, etc. It seems to be reasonable, therefore, to suppose that the failure of the graft can be attributed to the section of the nerves which reach the organ.

Crowe and Wislocki in experimental work on suprarenal glands in dogs attributed degenerative changes of the graft to the lack of nerve supply to it. We do not, however, fully concur with their opinion. Salzer and others also emphasize the independence of grafts from the nervous system. Manley and

Marine, in considering the question of whether or not secretory nerves were necessary to the gland, experimented on rabbits. They have demonstrated that such nerve fibers are not essential in order that thyroid tissue may exhibit the characteristic morphologic and physiologic changes known to be associated with great variations in functional activity. Other authors have also found that thyroid tissue may be readily transplanted in widely distant parts of the body. In our experiments on autoplastic transplantation we obtained good results in spite of complete dissection and removal of thyroids from their surrounding tissues. We cannot therefore consider that the failure of homoplastic transplantation of the thyroid is due to the section of its nerves.

Murphy tried to prove that a homoplastic or heteroplastic graft cannot develop indefinitely on its host. He discovered that the power of the organism to eliminate foreign tissue is due to organs such as the spleen or bone marrow, and that when these organs are less active (by removal of the spleen or the injection of benzol) a foreign tissue (tumor) can develop rapidly after it has been grafted. The grafts became absorbed from 12 to 15 days after the transplantation.

Hesselberg and Loeb attached a peculiar significance to lymphocytic reaction and to proliferation of connective tissue. They believe that the destruction of the thyroid tissue which occurs after the homografting is not caused by a direct primary disintegration or the solution of follicles, due to the action of substances circulating in the body fluids, but depends on the destructive activity of the lymphocytes, and of the connective tissue of the host. The former invade the follicles and destroy them directly; the latter surrounds and compresses, and thus destroys the follicles. Why do the lymphocytes and the connective tissues attack the homografts only, and not the autografts? It is well known that lymphocytes and connective tissue appear usually as the response to the reaction of foreign bodies. They may be commonly discovered around suture materials, such as silk and linen thread, etc., that have been used at operations. It is reasonable, therefore, to assume that homografts which do not take may act as foreign bodies.

The obstacle to success in homoplastic transplantation lies probably in the differences of biochemical factors between the animals. As Ullman expresses it, the cell protoplasm, specific for each organism, varies with the individual; there are as many protoplasms as there are individuals; in homoplastic transplantation the appearance in the body of a foreign protoplasm calls forth ferments into the circulation which destroy the transplanted tissue.

Stich enumerated the following factors as the cause of the destruction of the grafts: primary toxic action of the tissue fluid on transplanted organs; production of an antibody in the organism of the host through foreign albumin; and finally, starvation of the transplanted tissue, owing to the incapacity of the assimilation of materials that are necessary for its growth.

We are still ignorant of the means of eliminating these factors. Lexer (1914) showed that he had succeeded in overcoming some of the biochemical reactions between his animals by prolonged preliminary treatment of the host with tissues

and blood serum taken from the donor. He considered that the possibility of the improvement of homoplastic surgery was most hopeful along this path. Schöne believes that positive results may be obtained in animals through the same nourishment and the same mode of living for a long period of time. Sauerbruch and Heyde demonstrated that direct communication of blood vessels was proved between parabiotic rabbits, and that substances which are soluble in blood, iodine, strychnine, etc., and even bacteria, go from one animal to the other through newly formed vascular channels. The question of whether or not products of metabolism of one animal are pernicious to another has not been solved. Enderlen and his collaborators aimed to make the biochemical differences equal by direct blood vessel anastomosis in dogs, but they could not attain their object. In short, it seems to be most important to discover the method of equalizing as nearly as possible biological differences between donor and recipient.

CONCLUSION.

Our experiments on dogs showed that the thyroid gland which was autoplastically transplanted, by means of various methods of blood vessel anastomosis, could live in good condition and functionate favorably several months after the operation, even after the interruption of the circulation for $1\frac{1}{2}$ hours. They further showed that the circulation through the transplanted blood vessels as well as glands was as good as normal, and that permanent successful results of the homoplastic transplantation of the gland are as yet not possible.

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EXPLANATION OF PLATES.

PLATE 4.

FIG. 1. Photomicrograph of intact left thyroid of Dog 2. × 70.

FIG. 2. Photomicrograph of transplanted right thyroid of Dog 2 (autotransplant). Compare with the control in Fig. 1. × 70.

PLATE 5.

FIG. 3. Photomicrograph of intact right thyroid of Dog 3. × 70.

FIG. 4. Photomicrograph of transplanted left thyroid of Dog 3 (autotransplant). Compare with the control in Fig. 3. × 70.

PLATE 6.

FIG. 5. Photomicrograph of transplanted thyroid of Dog 9 (homotransplant). × 70.

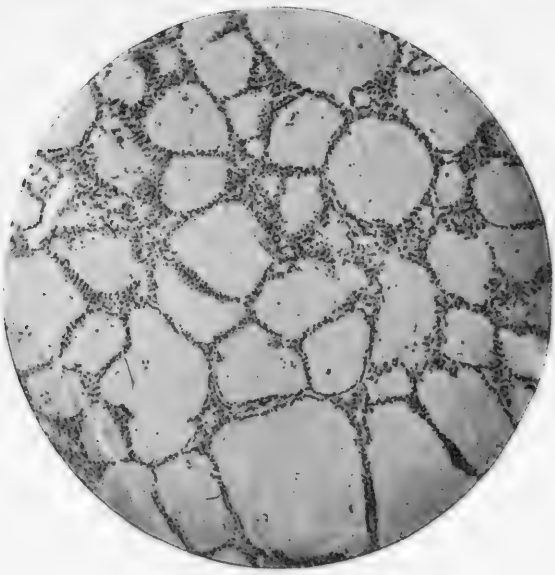

FIG. 1.

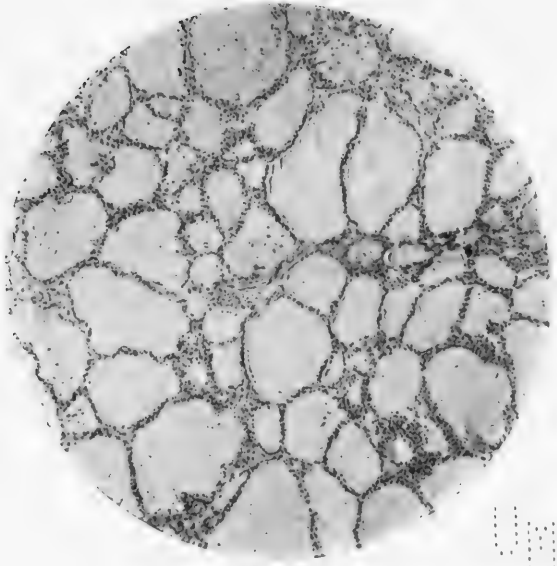

FIG. 2.

(Kawamura: Organ transplantation. I.)

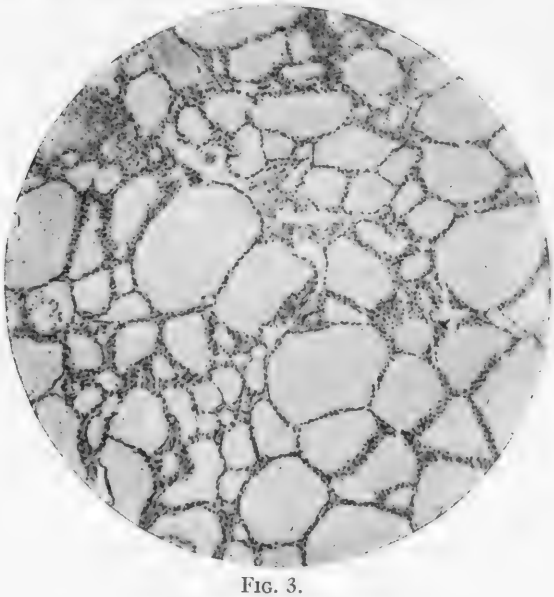

FIG. 3.

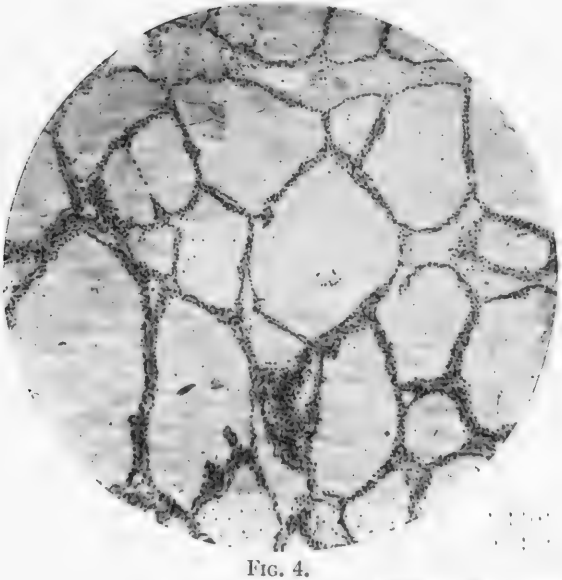

FIG. 4.

(Kawamura: Organ transplantation. I.)

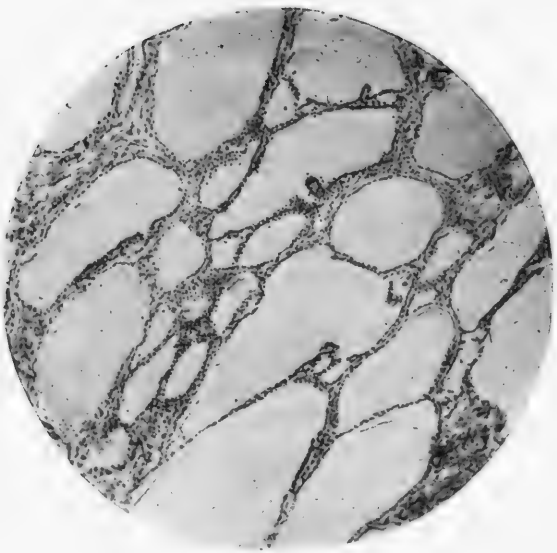

FIG. 5.

STUDIES ON ORGAN TRANSPLANTATION.

II. Transplantation of the Spleen with Intact Blood Supply.

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Plates 7 and 8.

(Received for publication, April 4, 1919.)

During the last 15 years organ transplantation has been studied extensively with especial reference to practical therapeutics and the biologic possibility of regeneration of transplanted tissues.

Many experiments have been reported on the ductless glands, and it has been affirmed that in these experiments the healing of the grafts depends largely on the site of the transplantation, and on the size of the transplant. As to the location of the graft, the opinions of authors differ widely. Cristiani, Ribbert, and others believe that the subcutaneous connective tissue is the best soil for the transplantation of the thyroid tissue; Payr preferred the spleen, while Kocher used the bone marrow. With regard to the size of the transplanting piece Cristiani, Ribbert, and Lubarsch claimed that numerous small fragments of thyroid tissue, the size of a wheat grain, are preferable. Payr, Kocher, von Bramann, and others advised the use of large grafts the size of a hazelnut or cherry because the larger portions undergo destruction.

Many instances of transplantation of the thyroid, parathyroid, ovary, and adrenals are found in the literature, and in some the results were favorable, although the percentage of success was small. On the other hand, reports of the transplantation of the spleen are rare, and the results have been unsatisfactory compared with those of the other ductless glands.

In 1916 Manley and Marine published an article on the transplantation of ductless glands. They transplanted six autografts and eighteen homografts of splenic tissue in the subcutaneous tissue of the abdomen, all of which were absorbed in 12 days. In 1917 they published another article on spleen transplantation. This time twelve attempts at homotransplantation and six attempts at autotransplantation were made on fifteen rabbits. The method employed was the same that was formerly used by them, and consisted of transferring small sections of the spleen of about 2 mm. into the subcutaneous fascia of the abdomen. None of their attempts at homotransplantation was successful beyond the usual results persisting for 2 or 3 weeks, common to all homografts. They ob-

tained only one successful permanent autotransplantation. The graft was removed at autopsy 325 days after transplantation and showed all the morphologic characteristics of a fully differentiated and functionally active spleen. The writers stated that considerable difficulty in spleen transplantation may be due to its complex anatomic structure.

The failure of transplantation of pieces of the organ, however, is partly attributable to insufficient blood supply in consequence of which the grafts are absorbed by degrees. Besides, it should be taken into consideration that small pieces, although successfully transplanted, are not always sufficient for the deficient functions. The transplantation of the entire organ by anastomosis of its blood vessels to suitable parts of the circulatory system can yield sufficient nutrition and probably also function.

Lüdke tried to transplant the spleen of dogs into the splenic vessels of a goat and a wether by blood vessel anastomosis. In all three instances there was faulty blood vessel anastomosis. He attributed the failure in the use of the technique to the unfavorable location of the spleen of these animals, and to the fact that the lumen of splenic vessels is too small. Lüdke, therefore, changed his first plan and transplanted pieces of spleen of rabbits or dogs into the abdominal cavity, the stomach wall, or into the spleen of rabbits, dogs, monkeys, goats, or wethers. The transplanted splenic tissue was observed for 4 weeks, but after 2 or 3 months no part of it was visible.

Carrel extirpated the spleen, washed it out with Locke's solution, and replaced it by blood vessel anastomosis in two dogs. One spleen necrosed and completely disappeared, due to the occlusion of the artery. The replantation of another spleen was successful, so that the anatomic condition of the vessels and of the organs was entirely normal about 8 months after the operation.

EXPERIMENTAL.

The entire series of our experiments was performed on dogs. There are usually two trunks of both artery and vein to the spleen from the gastrosplenic vessels. One pair enters the spleen in its lesser (left) end and another almost in the middle. The spleen was divided in two parts, corresponding to the stream district of these large branches, after the mattress sutures had been applied transversely on it. The half of the spleen which is nourished by the larger branches was used for the transplantation. The attached omentum was cut off after ligation. The splenic artery, vein, and nerves were dissected and

divided, Crile clamps being used. The caliber of the artery was hardly 1.5 mm. in diameter. The spleen was then removed, and wrapped up in a salt sponge. After a few minutes the spleen was replaced into the abdominal cavity, and its vessels were united as before, by end to end anastomosis. In one case an attempt was made to transplant the spleen into the neck. After the thyroid had been removed, the peripheral end of the splenic artery was united to the central end of the superior thyroid artery and the peripheral end of the splenic vein to the central end of the external jugular vein. In another case the spleen was transplanted into the renal vessels after nephrectomy. Most of the experiments were performed autoplastically, but in one instance the spleen from one animal was transplanted to another. The arterial suture was always difficult, due to the small caliber of the vessel. As soon as the clamps were unfastened, the bluish red collapsed spleen became very red, and its volume was increased. The circulation in the spleen was reestablished between 1 and 2 hours after its extirpation. The dissected omentum was reunited, and in a few cases the nerves also were sutured. Several days after the operation the condition of the transplanted spleen was ascertained by laparotomy. When the transplantation was successful, the other intact half of the spleen was removed and immersed in a jar filled with 10 per cent formaldehyde solution for microscopic examination, and the animal was observed further.

The protocol of a successful experiment is given below.

Experiment 1 (*Dog 1*).—Adult, mongrel bulldog, male; weight 16.9 kilos.

Oct. 5, 1918. Operated on under intratracheal ether narcosis. The spleen was exposed through a left rectus incision. Two rows of mattress sutures were placed in the middle of the spleen and the organ was divided between them. One half of the spleen, which was nourished with smaller branches of the splenic artery, was left in the abdominal cavity; the other half was used for replantation. After the dissection of the nerves and omentum, the artery and veins were cut so that this half of the spleen could be brought out extraperitoneally, apart from its connection. The spleen was wrapped up in a salt sponge for a few minutes and replanted into its original site by end to end blood vessel sutures. The arterial lumen measured hardly 1.5 mm. in diameter, and suturing was difficult. The venous suture, on the contrary, was easy. The clamp on the vein was first unfastened and then the clamp on the artery. The spleen, which during the operation appeared dark blue, and was contracted, became normal in color, and as-

TABLE I.

Summary of Experiments.

Experiment No.	Operation.	Duration of observation.	Results.			Remarks.
			Condition of blood vessels.	Condition of transplanted spleen.	Microscopic examination.	
	1918	*days*				
1 (Dog 1).	Oct. 5. Replantation of half of spleen. No suture of nerves. Nov. 27. Exploratory laparotomy. Extirpation of other intact half of spleen.	88	Arterial and venous suture in perfect condition. No thrombosis.	Capsule of spleen quite thick. Normal in color and consistency. Surface of section appeared normal.	Showed features of perfectly normal spleen.	Dec. 27. Dog fought with another dog and was severely wounded in several places. Jan. 1, 1919. The wounds proved fatal.
2 (" 2).	Oct. 7. Spleen which was divided in a proportion of one-third and two-thirds by mattress suture replanted, combined with nerve suture.	3	Thrombosis in central part of arterial suture. A small part at venous suture leaked.	Necrotic; softened.		Oct. 10. Died. A great deal of bloody fluid filled abdominal cavity.
3 (" 3).	Oct. 11. Dividing and replanting of the spleen. Reunion of omentum and nerves.	4	Blood vessels macerated.	" "		Oct. 15. Died. A great deal of bloody serous fluid in abdominal cavity.
4 (" 4).	Oct. 12. After removal of half of the spleen, half of spleen of Dog 9 was transplanted to this dog.	2	Transplanted splenic vein thrombosed.	" "		Oct. 14. Died. Hemorrhagic serous fluid in abdominal cavity.

5 (Dog 5).	Oct. 16. After removal of left thyroid gland, half of spleen transplanted to neck. Splenic artery united to thyroid artery and splenic vein to external jugular.	7	Both transplanted artery and vein thrombosed.	Necrotic; softened.		Circulation through spleen very good, but volume of spleen became much larger than before transplantation. After operation animal weakened by degrees and died Oct. 23.
6 (" 6).	Oct. 23. Replantation of half of spleen. Nov. 27. At exploratory laparotomy a large cystic tumor was found. Jan. 24, 1919. Extirpation of tumor.	93	Obstruction of artery at united part.	A cystic tumor about size of a child's head grew from replaced spleen. The cyst was filled with a muddy, purulent fluid.		Died during last operation; very emaciated.
7 (" 7).	Oct. 28. Replantation of half of spleen and reunion of cut end of omentum and splenic nerves. Nov. 27. Exploratory laparotomy. Excision of a small piece for microscopic examination.	56	Obstruction of transplanted vessels.	Exploratory laparotomy proved that replaced spleen diminished to size of tip of thumb. At autopsy no part of spleen was visible.	Capsule thickened. Peripheral zone of spleen stainable, but central part necrotic. Here and there formations of round holes of several sizes. Connective tissue, leucocytes, infiltration, and fat cells increased.	Dec. 23. Animal looked ill. Killed by cutting of femoral artery under general ether narcosis.

TABLE I—*Concluded.*

Experiment No.	Operation.	Duration of observation.	Results.			Remarks.
			Condition of blood vessels.	Condition of transplanted spleen.	Microscopic examination.	
	1918	*days*				
8 (Dog 8).	Nov. 25. After nephrectomy on left side half of spleen was transplanted to renal vessels. Dec. 9. Exploratory laparotomy. From transplanted spleen a small piece was cut off for microscopic examination. Jan. 25, 1919. Exploratory laparotomy.	61	Obstruction of artery at united part.	At second operation it was proved that the transplanted spleen kept its original size, but by incision no fresh blood flowed out. At third operation no part of spleen was visible.	Cells of capsule and subcapsular parts only were stainable.	The animal had been used also for experiments on transplantation of thyroid gland, and duodenectomy. Jan. 31, 1919. Died of intussusception in jejunum.

sumed its original size as soon as the clamps were removed. The circulation through the replanted spleen was reestablished after an interruption of 1 hour. No suture of the omentum or the nerves was made. After the circulation through the transplanted vessels as well as the spleen was assured, the adbominal wound was closed. The first incision was made at 9.14 a.m.; the last stitch was inserted at 11.20 a.m.

Nov. 27 (53 days after the first operation). An exploratory laparotomy was done. The weight of the animal was 17.7 kilos.; there were no marked adhesions; the omentum was normal. The replaced spleen had kept its original size and appeared entirely normal in color and consistency, but its capsule was thickened. The other intact half of the spleen was extirpated.

Dec. 27. The animal fought with another dog and was seriously wounded in the abdominal wall, back, and both hip regions.

Jan. 1, 1919 (88 days after the first operation). The dog died of his wounds.

Autopsy.—The wounds that had been made by fighting were found to be infected; there was no infection of the abdominal cavity. The replaced spleen looked very well. No thrombosis could be found in the splenic artery or vein; their lumina were patent throughout. The suture lines on the vessels could be found with difficulty. They were covered with normal epithelium and were smooth.

Microscopic Examination.—The capsule was quite thick in places as compared with the control specimen. Otherwise the structure of the pulp, the number and the size of the trabeculæ, and the Malpighian bodies were perfectly normal (Figs. 1 to 4).

Seven attempts at autoplastic transplantation and one attempt at homoplastic transplantation of the spleen were made, as shown briefly in Table I. Five of the seven autoplastic transplants were replanted to the splenic vessels, one to the neck, and one to the renal vessels. In two of the five replanted cases the dogs died in 3 and 4 days after the operation. At autopsy it was found that a great deal of bloody serous fluid filled the abdominal cavity, and that the spleen was necrosed, due to thrombosis. Two cases were examined 30 and 35 days after the operation by exploratory laparotomy. One half of the spleen was found intact in each case, but the other replaced half could not be seen. In one of these two instances there was about 1 liter of pale red, muddy, serous fluid in the abdominal cavity. A round, partly soft, partly hard cystic lump about the size of a child's head lay in the center of the cavity. This lump was surrounded by vast distended blood vessels and muddy gelatinous membrane. Later it was proved at autopsy that the lump was a cyst which had been formed by replanted spleen. The result of one operation (Experi-

ment 1), in which the spleen was replanted in a dog, was highly satisfactory. 88 days after the operation the spleen, as well as the united blood vessels, was in good condition. The spleen which had been transplanted into the neck was examined 7 days after the operation, and it was found that the whole tissue had undergone necrosis. The transplantation of the spleen into the renal vessels was troublesome, because the field of the operation was too deep and the caliber of the splenic artery was very small. Nevertheless, the immediate result of the operation was successful. As soon as the clamps were removed, the spleen became red and greatly distended. 14 days after the operation, when the exploratory laparotomy was made, the spleen retained its original size; but the color was pale red, and when it was incised dark blood came out. 47 days later the spleen had completely disappeared. The spleen which was grafted homoplastically was examined at autopsy 2 days after the operation. It was soft, dark red, and necrotic. The transplanted splenic vein was thrombosed. Hemorrhagic serous fluid filled the abdominal cavity.

DISCUSSION.

The results of the experiments immediately after operation were in all cases satisfactory. In spite of the interruption of the circulation from 1 to 2 hours after extirpation, the immediate circulation of blood through the transplanted spleen and blood vessels was favorable, but most of the spleen became necrotic or was entirely absorbed. The cause of this was the obstruction in the transplanted vessels, due to thrombosis. It is probably difficult to obtain good results by using so small a vessel as a branch of the splenic artery. Carrel has noted that such a small vessel cannot be sutured with many chances of success. As has been stated, all Lüdke's experiments failed.

Regardless of these difficulties, our successful case showed that such a highly differentiated, complicated organ as the spleen can be transplanted *en masse*, that it can keep permanently its normal structure, and probably also can functionate normally. In this instance the difference between the central and peripheral parts of the grafts, as Manley and Marine experienced by piecemeal transplantation, was not visible. This is scarcely to be expected because in trans-

planting by blood vessel sutures the nourishment of the transplant is maintained throughout.

In view of the fact that the spleen can survive even if the nerves are not united, the experiment demonstrated that nerves are not essential for the maintenance of grafts.

The neck, probably also the inguinal furrow, is not a favorable site for the experimental transplantation of the spleen by blood vessel suture, because after the suture of the fascia and skin the more or less distended spleen is compressed, and, consequently, disturbances of the circulation through the graft may occur.

SUMMARY.

Seven autoplastic transplantations and one homoplastic transplantation of the spleen of dogs were made. One autotransplantation was successful, the gland being normal at the end of 88 days.

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EXPLANATION OF PLATES.

PLATE 7.

FIG. 1. Photomicrograph of the intact portion of the spleen of Dog 1. × 50.
FIG. 2. Photomicrograph of the same specimen as Fig. 1. × 125.

PLATE 8.

FIG. 3. Photomicrograph of the transplanted portion of the spleen of Dog 1. × 50.
FIG. 4. Photomicrograph of the same specimen as Fig. 3. × 125.

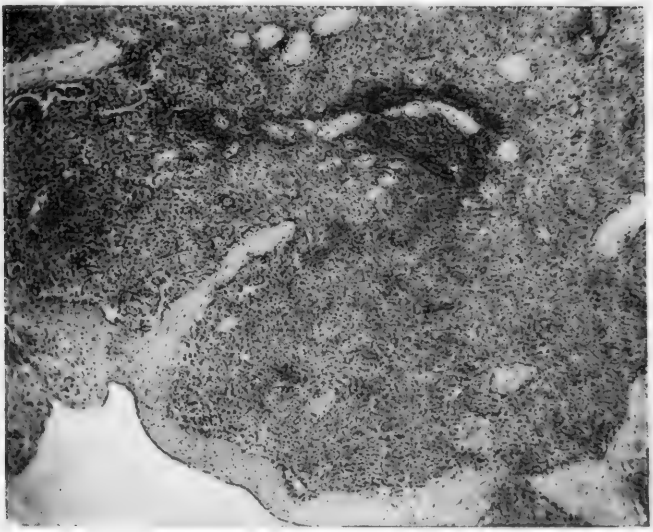

FIG. 1.

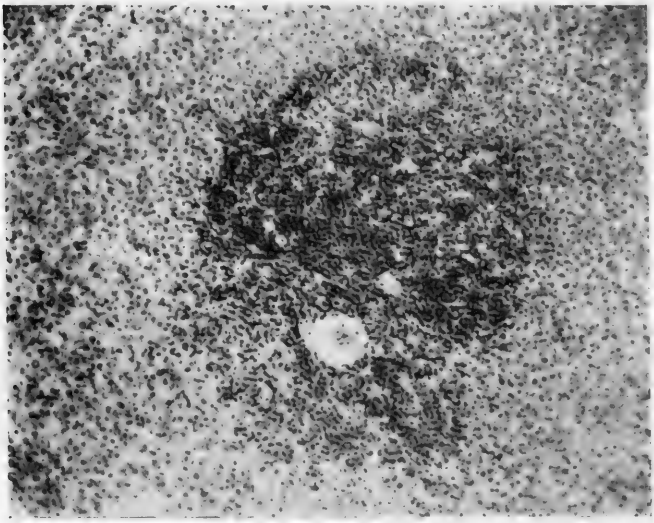

FIG. 2.

(Kawamura: Organ transplantation. II.)

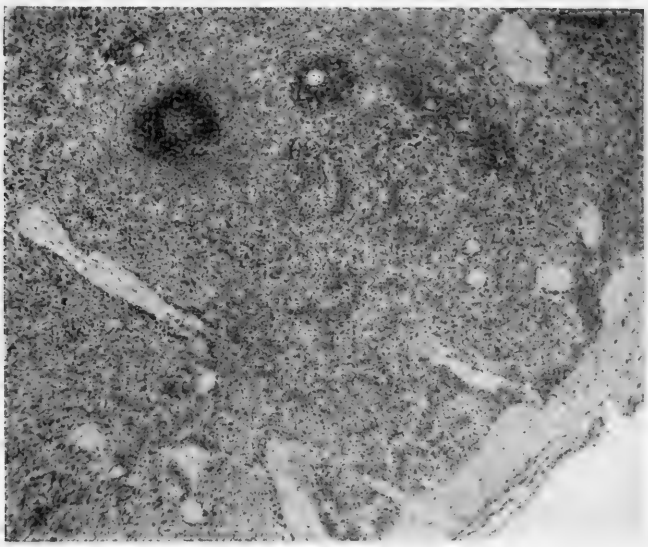

FIG. 3.

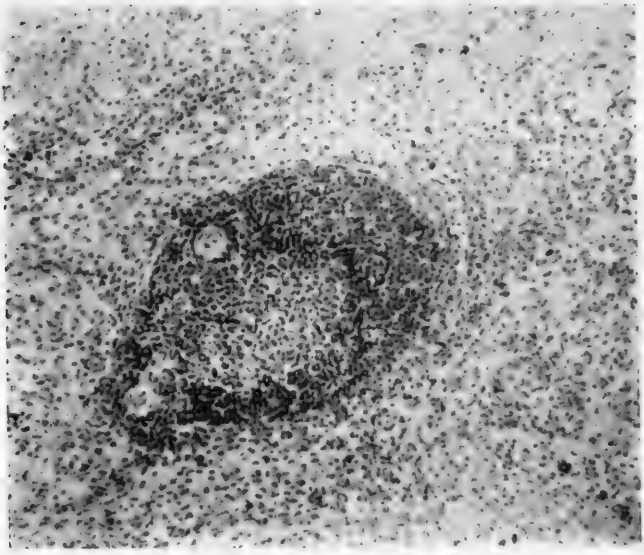

FIG. 4.

(Kawamura: Organ transplantation. II.)

THE EXPERIMENTAL PRODUCTION IN DOGS OF EMPHYSEMA WITH ASSOCIATED ASTHMATIC SYNDROME BY MEANS OF AN INTRATRACHEAL BALL VALVE.

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Plates 9 to 11.

(Received for publication, April 8, 1919.)

While performing certain work upon dogs with a plethysmograph devised by Chillingworth and Hopkins,[1] varying degrees of emphysema of the lungs were noted by them when the animals were autopsied. The plethysmograph was so constructed that the animal's entire body was subjected to a negative pressure by rarefying the air in the interior. The trachea was, however, connected to a tube leading to the outside air, so that the intratracheal and intrapulmonic pressure was that of the atmosphere and so much in excess of that of the extrathoracic pressure within the plethysmograph that the lungs and thorax were distended. The consequent rupture of the alveolar walls was controlled by the differences in these pressures which could be adjusted to a nicety.

The emphysema produced by this negative pressure method was acute and afforded no opportunity for clinical study, nor did this method of production simulate the factors concerned in pulmonary emphysema in man. In order, therefore, to produce a more gradual emphysema and at the same time to simulate more closely the mechanism by which this condition is brought about in man, the writers undertook to devise a method which would accomplish this purpose. Since in cases of emphysema and asthma the respiratory difficulty is mainly one of expiration, our efforts were directed to a

[1] Chillingworth, F. P., and Hopkins, R., *J. Lab. and Clin. Med.*, 1919, iv, No. 9 (in press).

duplication of this condition of altered respiration with a view to determining whether emphysema could be produced. It was found possible to accomplish this by introducing a ball valve into the trachea, which would prolong and partially obstruct expiration without impeding inspiration.

A series of valves was constructed which offered varying degrees of expiratory impediment. In the early experiments it was found that many of the valves occasioned either too great or too little expiratory obstruction. Many trials were necessary, therefore, before the correct adjustment was obtained. With properly adapted valves it has been possible to produce at will mechanical emphysema varying from acute to chronic. These valves (Fig. 1) were made of brass tubing 4 cm. long, varying in outside diameter from 8 to 13 mm. and having an inside diameter or bore of 5 to 7 mm. Upon the upper end and outer side of the valve at opposite points were soldered two narrow flat copper wings which projected about 1 cm. beyond the end of the valve. These wings when sprung slightly outward aid in maintaining the valve in position and prevent its expulsion by impinging against the mucosa of the trachea. Into each end of the cylinder a collar was inserted and these collars when so placed formed two small shelves, an upper and a lower, which projected a slight distance into the lumen of the tube. Small rectangular channels were cut into the shelves for the passage of air. The number and size of the channels were graded according to the air obstruction desired. A gutta-percha ball was placed between the two collars which during inspiration was sucked to the lower valve seat leaving the lumen of the tube open above and permitting free ingress of air through the several channels in the lower collar. During expiration the ball was raised against the upper collar, closing the upper lumen of the valve, thus permitting egress of air only through the channels, the narrowness of which offered the desired obstruction. The degree of this obstruction to expiration was graded to the number and width of the openings in the upper collar which varied from four channels with a width of 1 mm. and a length of 4 mm. down to collars carrying but two channels, having a width of 2 mm. and a length of 2 mm. The lower collar carried sufficient channels to insure practically normal inspiration.

Method of Introducing the Valve.—To insert the valves, the dogs were given a moderate dose of morphine, then deeply anesthetized with ether and placed on the belly. The tongue was pulled forward with a retractor exposing the glottis; with a long pair of forceps the lower lip of the glottis was pulled forward and the valve inserted in the trachea below the vocal cords. The size of the valve employed was selected in accordance with the size of the trachea of the animal used. The valve was carried into position by a wooden probang which entered the valve lumen readily and served as a director. When the valve was placed in position the director was withdrawn, thus completing the operation. A skiagraphic print of a valve in position is shown in Fig. 2. This particular valve had been in position 20 days.

Upon recovery from the anesthetic and morphine many of the animals for the first few hours exhibit symptoms of marked tracheal irritation characterized by violent coughing and attempts to expel the valve. Within 24 hours this phase is replaced by utter lack of local irritation; in fact in chronic emphysematous animals external manual pressure upon the trachea and valve elicits little or no response. The probable explanation of this is no doubt analogous to the adaptability of man to the tracheotomy tube. There is present in both instances a primary irritation by the foreign body, but this stage is followed by a loss of reflex stimulation; the constant contact exerts a uniform degree of pressure upon the inner coat of the trachea, thereby probably producing a pressure anesthesia upon certain terminations of the laryngeal nerves. The irritation from the valve in the dog is less than that from the tracheotomy tube in man because there is less motion or friction between the cylinder and the trachea, and, again, the air inspired is first passed through its normal route and thereby filtered and warmed as contrasted with air received directly from the atmosphere with the tracheotomy tube. The bechic spasm is probably in part relieved by the coat of mucus formed below the valve.

For the purpose of studying the carbon dioxide content of the blood and alveolar air under circumstances simulating asthma,

Friedman and Jackson[2,3] produced expiratory obstruction in dogs. The obstruction was occasioned by employing a one-way valve in conjunction with a T-tube in the trachea. The valve was arranged so that a screw attachment would control the degree of expiratory obstruction. Their experiments were conducted with dogs under anesthesia so that the valve necessarily acted for a short duration of time, with the animal breathing through the tracheal tube and in an unconscious state. With the ball valve arrangement the dogs are observed after anesthesia over prolonged periods of time and with the source of air through the upper respiratory passages as in normal dogs. In conjunction with their observations an acute emphysema of the lungs was noted by them and described by Fraser.[4] While a certain relationship exists in the two procedures the intratracheal ball valve permits obviously of observations under distinctly different conditions.

The present study has been based upon the observation of twenty-five dogs of varying sizes, ages, and types. The period has varied from 24 hours to 3 weeks from the time of valve introduction until autopsy. A routine observation of the dogs after the first 24 to 36 hours shows the animal apparently normal with the exception of expiratory dyspnea. This dyspnea varies from a mild to a marked degree depending upon the expiratory interference offered by the valve inserted. The presence of the valve in no way interfered with the health of the animal. The appetite was normal, and in fact with dogs that were received in a poor condition an actual gain of weight often occurred, due to proper food and care. There is a gradual enlargement and prominence of the thoracic cavity. In due course of time percussion elicits a hyperresonant note and the various features of emphysema are noted. While it might appear that accumulations of mucus would interfere with the valve action, such in reality does not occur because of the forced type of valve expiration. The valve action is readily heard with the stethoscope over the trachea. The most striking observation is the marked expiratory dyspnea noted after exercise or upon the brief inhalation of such

[2] Friedman, E. D., and Jackson, H. C., *Arch. Int. Med.*, 1917, xix, 767.

[3] Friedman, E. D., and Jackson, H. C., *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 73.

[4] See Friedman and Jackson,[2] p. 768.

mild respiratory irritants as dilute ammonia fumes or tobacco smoke. The manifestations simulate closely those of asthmatic paroxysms in man even to the occurrence of the wheezing sounds.

Professor George S. Bel of the Department of Medicine was requested to examine one of the animals during a paroxysm occasioned by mild exercise. No information of any sort was given him and the following was the result of his examination: On inspection, the head was pushed forward, the thorax distended, and slight cyanosis was observed in the tongue and gums. The respirations were slow with marked prolongation of the expiratory phase. Percussion elicited a hyperresonant note over the lung area of both sides. Upon auscultation, sonorous and sibilant râles were heard over both lungs. The expiratory sounds were markedly prolonged and accompanied with wheezing. The heart was irregular and there was noted an accentuation of the second sound over the pulmonic area. Diagnosis: Marked emphysema with accompanying asthmatic attack.

In connection with other observations graphic records of respiration were carried out. The pneumograph of Ellis was employed in conjunction with a modified Marey tambour, the transmitting tube of which was sufficiently rigid to withstand all possible pressure variations. The records were found practically consistent in their curve, the down-stroke recording inspiration and the up-stroke expiration. The series of respiratory tracings shown in Figs. 3 and 4 demonstrate typically the loss of respiratory balance concomitant with pulmonary emphysema. In Fig. 5 is seen the normal respiratory curve recorded during rest, the rate being fourteen per minute. The passive type of normal expiration is clearly shown in this tracing. Fig. 3 is a record of valve respiration made after the valve had been in position 48 hours, with the animal at rest, the rate being ten per minute. While the rate of respiration is slightly slower than that in Fig. 5, the depth has been relatively increased so that this change is of no consequence. The striking difference between the normal respiration and the valve respiration as shown by the records is the increased length of time of expiration caused by the ball valve's changing the expiratory phase of respiration from passive to non-passive. Fig. 4 of this series was recorded after mild exercise which consisted in running the dog about for a few minutes.

This tracing exhibits several interesting new phases. In the first place the rate of respiration has not been increased; if anything it is slower than in Fig. 3. Normally, exercise increases the rate and depth of pulmonary ventilation, but with the valve in the trachea the respiratory rate does not increase with exercise; in fact it is mechanically slowed, but correspondingly increased in depth. After exercise the expiration is markedly forced and prolonged, and it would seem that the forced expiration is responsible for the decrease in the rate. In a few instances attacks were precipitated by the temporary inhalation of dilute ammonia fumes or tobacco smoke, and in these cases the asthma-like paroxysms were extreme. The mechanical interference with expiration results in a change in the nervous regulation which more than counterbalances the chemical stimulation from exercise. The points marked x in Figs. 3 and 4 indicate the impinging of the ball against the upper seat and it can be readily seen that the remainder of the expiratory phase which occurs between this point and the beginning of inspiration is prolonged and forced. The inspiratory act is delayed and inhibited by the slow deflation of the lungs until sufficient collapse has taken place to produce the necessary stimulation of the pulmonary branches of the vagus.

Pathology.—All the animals were autopsied and the lesions produced observed, with special reference, however, to the lungs. As death was produced in the animals by anesthesia and possible changes might occur in the lungs as a result of this, certain precautions were carried out in order to reduce to a minimum the respiratory struggle. In certain instances death was produced by toxic doses of nicotine, but as a routine procedure it was our custom to give a large dose of morphine, and after 2 hours death was produced by a small amount of ether. Morphine has the double value in that it not only prepares the animal for ether, but it also causes a marked slowing of respiratory movements, thereby minimizing distention of the alveoli which might accompany death from ether anesthesia. The gross appearance of the experimental lungs presented in general the common picture observed in human emphysema. Its production was found to vary in extent and degree according to the type of valve employed and the duration of the experiment.

Upon opening the thoracic cavity the lungs *in situ* were distended and showed no tendency to collapse. The organs were usually quite pale in color indicating a diminution of the blood supply to the parts. After removal of the lungs distinct vesicular wall rupture was noted as evidenced by numerous bullæ-like clusters present over the surface but most marked along the lung borders. Fig. 6 demonstrates the ordinary gross aspect of the pluck wherein the period of time was 7 days. In animals where the experiment extended over a short period and in which the valve employed was markedly obstructive, the emphysematous tissue was not of the pale color noted in experiments of longer duration and with less obstructive valves. In these short period experiments small areas showing hemorrhagic extravasations were occasionally noted. Such extravasations were attributed to rhexis of the smaller vessels due to the intense pressure occasioned by the alveolar distention and consequent vascular disturbance. Similar hemorrhagic extravasations were noted by Fraser[4] in the experiments of Friedman and Jackson,[2] wherein an acute emphysema had been produced by their method. Occasionally at autopsy the experimentally produced emphysematous lung revealed discrete and sharply defined small consolidated areas, distinctly red in color, which proved to be secondary lobular pneumonia.

The trachea showed but slight if any pathological changes in the area from which the valve was removed. At the locations of the wings or projections which retained the valve in position varying degrees of inflammation from intense congestion to pressure necrosis of the surface with the presence of a slight amount of fibrinopurulent exudate were noted. This lesion was superficial and at no time did extensive sloughing occur. In the trachea below the valve and in the larger bronchi a slight amount of glary mucus was usually present.

Microscopic examination revealed the usual histopathology of emphysema in all the lungs studied. The walls of the air cells were abnormally thin. The interalveolar septa were in many instances ruptured giving rise to intercommunicating compartments of a few to many air cells. The larger vessels of the interstitial tissue were engorged as a result of the capillary obstruction. No evidences of

hyperplasia of connective tissue were noted. Fig. 7 is representative of the usual histopathological picture presented.

Weigert's elastic tissue stain demonstrated the ruptured fibrils. In some areas these were greatly attenuated and broken, while in others coarser fibrils were abruptly ruptured and the ends curled.

Control Animals.—As a foreign body *per se* in the trachea might have been considered sufficient to produce the results obtained, animals in which a false valve was inserted were observed and studied. The control valve differed from the others only in that its inner collar was not slotted and that it contained no gutta-percha ball. These animals after primary local irritation had passed were normal in every respect and could not be differentiated from dogs in which no valve had been introduced. They could growl and bark at will. Infrequently, a slight movement of the control valve in the trachea following marked exercise would cause a mild degree of coughing and an attempt to expel the valve. Autopsy of such control animals showed no experimental emphysema. Here and there on the lung edges slight emphysema was noted. In this connection, however, it must be stated that such a picture is observed in a considerable proportion of dogs employed in routine physiological laboratory work, especially if death has been produced by a general anesthetic.

DISCUSSION.

Emphysema in man is commonly caused by various conditions wherein there is an increase in the amount of residual air and some interference of the proper expiratory air egress. These factors may be permanent or they may be occupational or habitual. For example, emphysema is produced in those whose occupation is the blowing of wind instruments or glass blowing and the like. In these instances very full inspirations occur which include the complemental intake and are accompanied by difficult prolonged expiratory output through the instrument employed.

In certain bronchial conditions with proliferative or exudative thickening of the bronchial tubes and consequent narrowing of their lumina, it is probable that the condition may cause little if any impediment to the intake but may result in marked expiratory ob-

struction and thereby produce emphysema. The explanation of this apparent anomaly is to be found in the well known physics of respiration which have already served to explain the difficult expiration of asthma. This mechanism is clearly expressed by Starling[5] as follows:

"There is, however, a difference in the mechanical conditions of the bronchi during the two phases of a respiratory movement. Normally the elastic structure of the lungs is drawing upon the bronchial wall, tending to maintain it patent, and so opposing the action of the bronchial muscle. During inspiration this expanding force is increased, so that in the presence of bronchial constriction the access of air is rendered the easier, the more powerful the contraction of the inspiratory muscles. In expiration all parts of the lung collapse, drawing with them the chest-wall; the pull of the lung tissue on the bronchial wall is lessened, but is still present. If, however, the expiratory muscles contract vigorously, the intrapleural pressure becomes positive, and the pull of the lung tissue on the bronchial walls is changed into a pressure tending to obliterate their lumen and so impede the out flow of air."

In addition to the above factors, it should also be remembered that at the beginning of the inspiratory act there is a rarefaction of the remaining air in the alveoli, which facilitates the ingress of air into the lungs, whereas the expiratory act is directed against atmospheric pressure.

The coughing associated with chronic bronchial lesions would seem to be a logical contributory cause in the production of emphysema just as in whooping cough. In coughing, complemental air is inspired and forcible expirations are impeded by a narrowed opening of the glottis.

The occurrence of asthma-like attacks following exercise of the animals is noteworthy, especially as asthma and emphysema are so often associated in man. It would seem that in instances wherein prolonged interference with expiration exists and under certain circumstances which demand a greater oxygen intake or carbon dioxide discharge, asthmatic seizures are precipitated. In support of this view Hoover and Taylor,[6] Friedman and Jackson,[2,3] and others have

[5] Starling, E. H., Principles of human physiology, London, 2nd edition, 1915, 1048.

[6] Hoover, C. F., and Taylor, L., *Arch. Int. Med.*, 1915, xv, 1.

shown that such attacks are precipitated by an undue amount of alveolar carbon dioxide. The exact phenomena of this process seem as yet not definitely settled. In our experimental animals moderate exercise was employed to provoke the asthmatic syndrome, but in man emotions such as excitement, mucus accumulations, and other factors might provoke a similar paroxysm. With valves causing undue expiratory obstruction and consequent insufficient carbon dioxide discharge, asthma-like attacks can be produced experimentally immediately after insertion. As such a valve occasions a persistent obstruction which cannot be relaxed like the bronchial spasm, the valve must be removed or death will ensue. In man so called bronchial asthma *per se* is sometimes attributed to bronchial constriction alone, and it is considered that paroxysms can occur without, perhaps, any apparent exudation or thickening of the mucosa; however, even in these cases the spirals of Curschmann are usually present.

It has not been our purpose in this paper to consider such forms of emphysema as the senile and compensatory or that noted in experimental anaphylaxis by Auer and Lewis[7] and others, nor was it our intention to discuss fully bronchial or neurotic asthma. Nevertheless certain data bearing upon these may be observed by means of the experimentally produced emphysema herein described.

Our observations upon the production of emphysema in the dog have led us to believe that certain factors play an important part in its causation in man which may be enumerated as follows: (1) the degree and duration of the impediment to expiration; (2) the histological structure of the alveolar walls, including the character of the capillary vessels and of the elastic fibrils especially with reference to age; (3) the degree of bronchitis established; and (4) the efficiency of cardiac action. These factors combined or in part seem to form the basic units in the production of pulmonary emphysema.

CONCLUSIONS.

1. Experimental emphysema can be produced in dogs by impeding expiration by means of a properly constructed ball valve introduced into the trachea.

[7] Auer, J., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 151.

2. This method affords a practical means of study of various physiological, pathological, and clinical aspects of emphysema.

3. The experiments demonstrate that any lesion or factor capable of interfering sufficiently with proper air expulsion yet permitting of sufficient air intake will occasion emphysema.

4. Attacks simulating those of asthma are occasioned in experimental emphysematous animals after exercise as well as during and following inhalation of mildly irritant gases.

EXPLANATION OF PLATES.

PLATE 9.

FIG. 1. Detailed drawing of longitudinal section of valve showing upper and lower collars with channels for passage of air. The ball is seen in a neutral position between the collars; the position in inspiration and expiration is indicated by the dotted circles. On the head end of the valve are illustrated the wings which aid in maintaining the valve in position.

FIG. 2. Dog 1. Roentgenogram of valve in position in the trachea. The trachea and larynx appear as a white tubular space above the valve. The cervical vertebræ have been rotated and extended so as to demonstrate the valve and trachea. This valve had been in position 20 days.

PLATE 10.

FIG. 3. Dog 2, female. Record of valve respiration during rest, 48 hours after valve insertion. The points marked x in this tracing and in Fig. 4 indicate the impinging of the ball against the upper valve seat. Between this point and the beginning of inspiration it will be noted that the expiration is forced and prolonged. Time 5 seconds.

FIG. 4. Dog 2. Record of valve respiration immediately following mild exercise. The extreme expiratory difficulty resulting therefrom is markedly increased.

FIG. 5. Dog 2, weight 12.33 kilos. Respiratory record of normal animal at rest. Time 5 seconds.

PLATE 11.

FIG. 6. Dog 3. Photograph of pluck, illustrating gross appearance of pulmonary emphysema. Distention of lung and bullæ-like compartments can be noted. Hemorrhagic extravasations are present on the surface of one of the lobes. This experiment extended over a period of 7 days.

FIG. 7. Microphotograph of area of emphysema demonstrating thinning of alveolar walls and rupture of some of the walls forming communicating compartments. Congestion is present in the larger blood vessels. × 45.

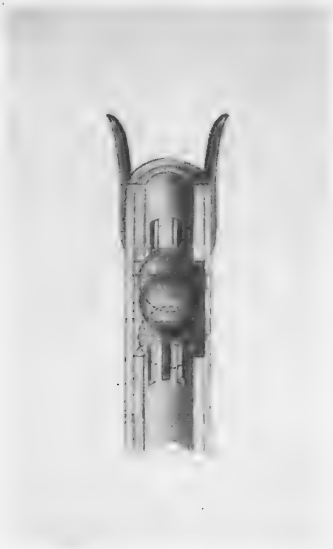

FIG. 1.

FIG. 2.

(Harris and Chillingworth: Emphysema with asthmatic syndrome in dogs.

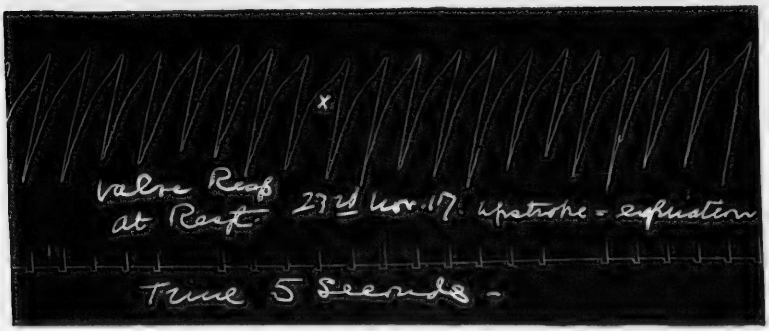

FIG. 3.

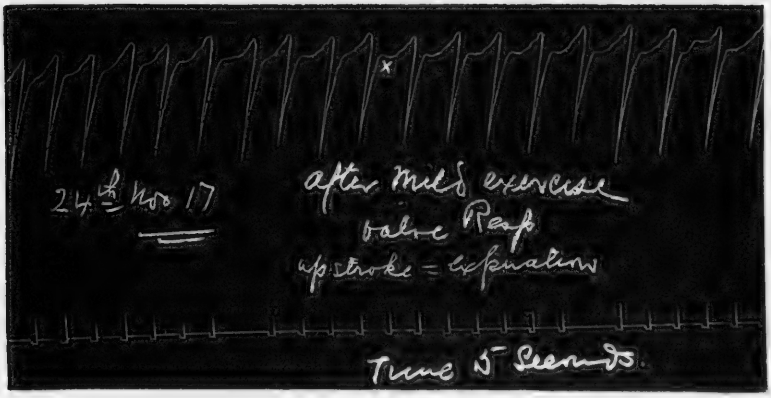

FIG. 4.

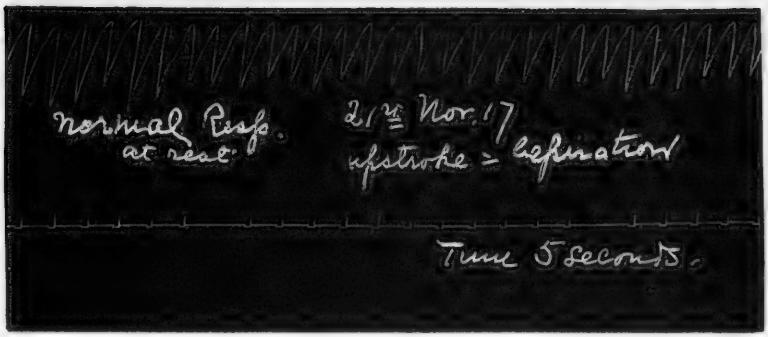

FIG. 5.

(Harris and Chillingworth: Emphysema with asthmatic syndrome in dogs.)

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FIG. 6.

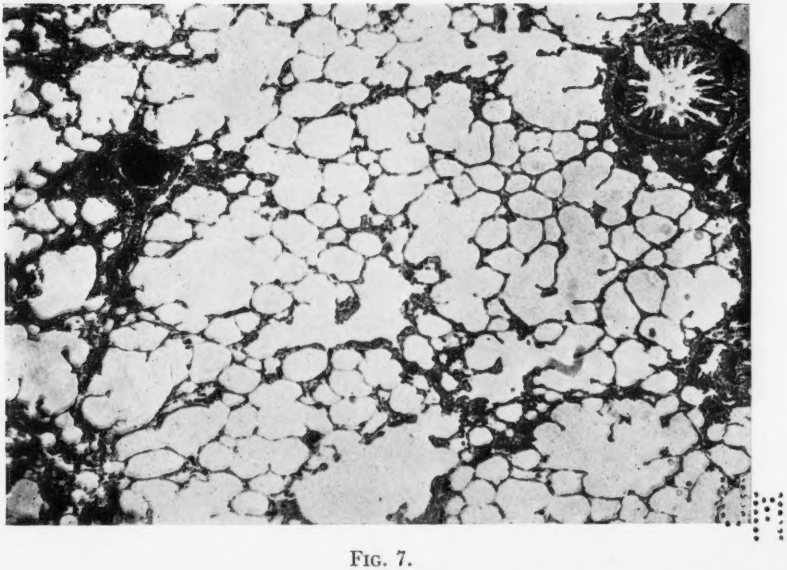

FIG. 7.

(Harris and Chillingworth: Emphysema with asthmatic syndrome in dogs).